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EVALUATION OF INSECTICIDE-TREATED NETS AGAINST CX. QUINQUEFASCIATUS MOSQUITOES IN PUNJAB, PAKISTAN

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Abstract

Background and objectives: *Mosquitoes are well-known vectors of numerous diseases on a global scale. Culex quinquefasciatus, a widely recognized urban mosquito species, serves as a carrier for filarial parasites and various arboviruses. In the current study, the effectiveness of DawaplusR 2.0 ITNs against Cx. quinquefasciatus was assessed.*

Methods: *The larvae were collected from various habitats in the districts of Narowal and Gujrat, Punjab, Pakistan. The larvae were reared in the laboratory under the standard rearing conditions. For the purpose of assessing mosquito resistance towards ITNs, adult female mosquitoes were used following the WHO tunnel bioassay. The calculation was done to determine the percentages of mortality, passage, and blood-feeding inhibition.*

Results: *In comparison to the control group, the percentage of mortality, passage and blood-feeding inhibition shows that the ITNs can provide protection against mosquitoes. However, their effectiveness in offering protection against the resistant populations was limited.*

Interpretation & conclusion: *The findings of this study suggest that the ITNs employed exhibited a general reduction in the mosquito population. Depending on the site of collection, the effectiveness of nets varies. This study highlights the importance of considering contextual variables when implementing mosquito control interventions. More research and surveillance are needed to develop effective approaches for managing Cx. quinquefasciatus in different areas.*

Keywords: *Insecticide-treated Nets (ITNS), Insecticide Resistance, Mosquito-Borne Diseases, Mosquito Control, Sustainability*

INTRODUCTION

Mosquitoes are the primary vector for several diseases that significantly influence human health. Malaria, dengue fever, and the Zikaa virus are just a few of the mosquito-borne diseases that continue to be a global health crisis (1). Cx. quinquefasciatus is a globally distributed mosquito species that is known as the primary vector of filarial parasites and several arboviruses, including West Nile Virus, Japanese encephalitis virus, and Rift Valley fever virus (2, 3). Most diseases that are caused by mosquitoes, currently have no viable vaccines. Therefore, the World Health Organization (WHO) has declared that changing behavior, raising public awareness, and managing the environment are the most important preventive approaches against mosquito-borne diseases (4, 5). Mosquitoes can be managed in communities with the help of chemical and biological approaches. There are many methods for warding off mosquito bites, including screening one's home with nets, mats, insect repellent cream, coils, etc. Other effective techniques in mosquito control include locating and eliminating breeding locations, managing the surrounding environment, and raising public awareness (6). The mechanical way to protect oneself from mosquitoes is by the use of "bed-nets", as it acts as a barrier from external vectors. Insecticide treated nets (ITNs) are able to create an additional chemical barrier, which kills any mosquitoes that come into contact with the net. This increases the efficacy of bed-nets and decreases the likelihood of subsequent human vector encounters as a result of the induced death (7). ITNs are required not only to act as physical barrier against bites but also to

remain effective against mosquitoes even after the user has washed them multiple times (up to 20 times) (8). The WHO has identified four probable types of ITNs which include simply pyrethroid, pyrethroid with insect growth regulators, two kinds of synergists that combine with pyrethroids i.e., piperonyl butoxide (PBO) and non-pyrethroid insecticide (with distinct form of action). Currently, WHO only recommends the usage of pyrethroid and pyrethroid-PBO (9, 10). ITNs have appeared to be one of the most efficient and successful methods for limiting disease transmission by mosquitoes (6). There has been a significant reduction in the number of cases of diseases caused by mosquitoes in a great number of nations as a result of widespread distribution and marketing of ITNs (11). The success of ITNs in such areas has led to a large drop in mortality rates, particularly among the populations that are more susceptible to the effects of the disease like young children and pregnant women (12). In spite of the fact that they have been demonstrated to be effective, there are still a number of obstacles that must be overcome in order to maximize the effects of ITNs. These hurdles include pesticide resistance, universal availability, behavioral variable, and sustainability (13, 14). In the context of effectiveness to improve public health, the rate at which ITNs are successful in preventing the spread of diseases carried by mosquitoes is a vital factor (14). The focus of this research was to evaluate the performance of deltamethrin-treated nets which belong to class pyrethroids in various areas of the Narowal and Gujrat districts. The WHO tunnel bioassay was done to measure the fatality rates, mosquito penetration and blood-feeding habits. The purpose of this study was to determine the effectiveness, limitations, and potential strategies for enhancing the impact of ITNs against Cx. quinquefasciatus.

MATERIALS AND METHODS

DESCRIPTION OF THE STUDY AREA

Mosquito samples were collected from multiple sites within two districts of Pakistan, Gujrat and Narowal. The details of the collection sites from district Narowal and Gujrat are explained in Fig. 1 and Table I.

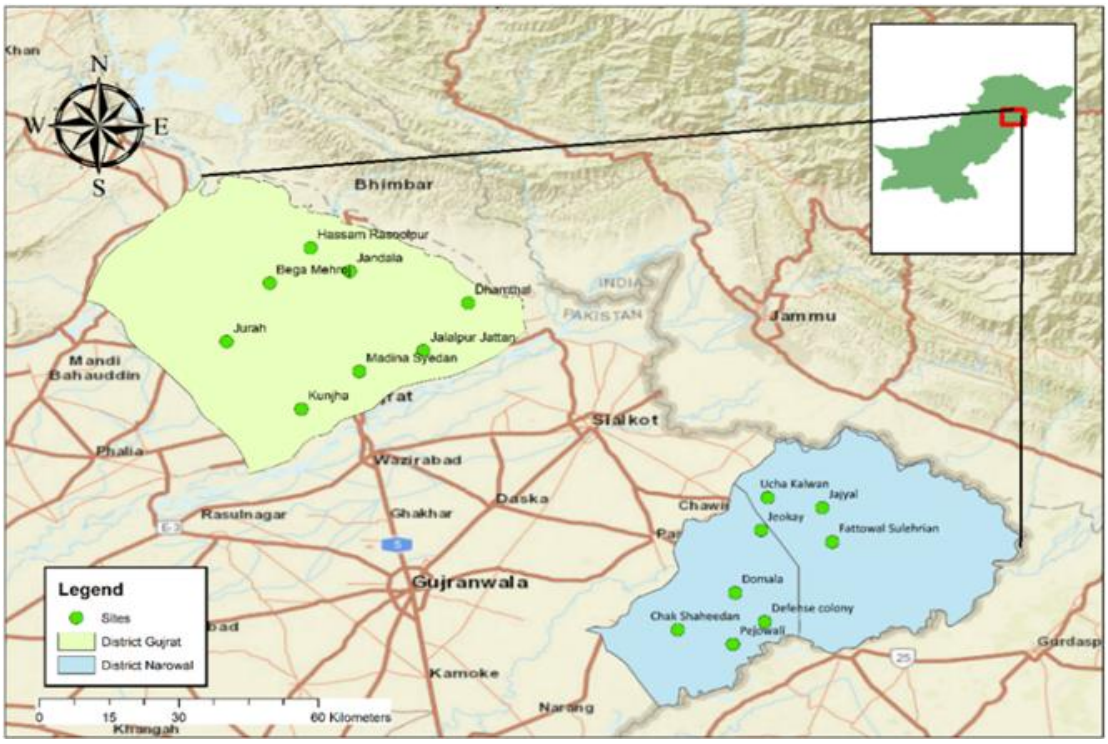

Fig. 1. The map showing the locations of mosquito collection from Narowal and Gujrat, Pakistan

SAMPLE COLLECTION

DISTRICT NAROWAL

The geographical area of the district of Narowal is 2,337 km2. In the northwest, it borders Jammu and Kashmir and the Sialkot district; in the south, it borders Sheikhupura and Amritsar and in east, it borders Gurdaspur district (17).

Table I. Description of collection sites of Cx. quinquefasciatus from Narowal and Gujrat, Pakistan

District	Locations	Sites	Average temperature	Average rainfall	Major crops	Reference
Narowal	Defense Colony	N-1	Summer: 29.6 to 42.2°C Winter: 4 to 21°C	21.98 mm	Wheat, Rice, Sugarcane, Maize, Pulses, and various vegetables	(15)
	Damala	N-2				
	Chak Shaheedan	N-3				
	Pejowali	N-4				
	Jeokay	N-5				
	Ucha Kalwan	N-6				
	Jajyal	N-7				
	Fattowal	N-8				
	Sulehrian					
Gujrat	Madina Syedan	G1	Summer: 45°C Winter: 2°C	67mm-75mm	Wheat, Rice, Maize, Mustard, Sunflower, Moong, Mash, and various vegetables	(16)
	Kunjha	G2				
	Jalalpur Jattan	G3				
	Dhamthal	G4				
	Jurah	G5				
	Bega Mehroj	G6				
	Jandala	G7				
	Hassam Rasoolpur	G8				

DISTRICT GUJRAT

Gujrat district occupies more than 3192 square km area. The districts of Jammu & Kashmir, Jhelum, Sialkot, Gujranwala, and Mandi Bahauddin border the district to the northeast, northwest, east and southwest respectively (18).

COLLECTION AND REARING OF CX. QUINQUEFASCIATUS

The larvae of Cx. quinquefasciatus were collected from various locations in Narowal and Gujrat as shown in Fig. 1. By using a dipper with a capacity of 350ml, larvae were collected from ponds, sewage water, clogged gutters, and water tanks.

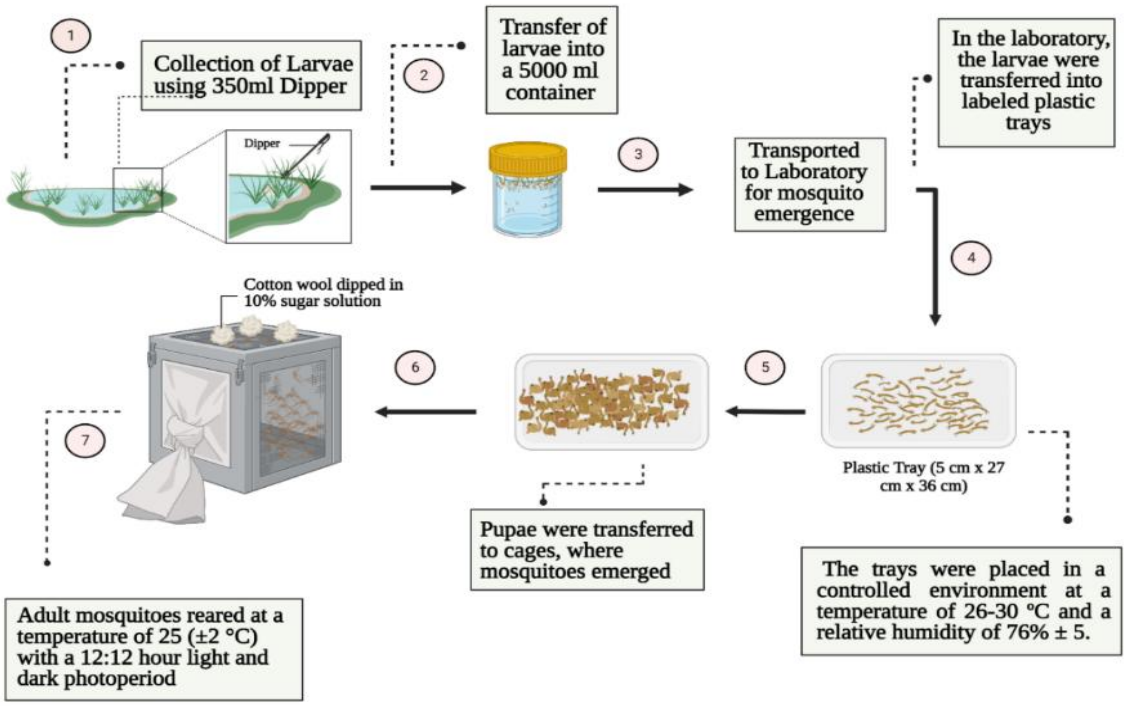

Fig. 2. A schematic presentation of collection and rearing of Cx. quinquefasciatus in the laboratory.

A 5000ml container was then used to carry the larvae to the laboratory for rearing purpose. The larvae were transferred into 5 cm x 27 cm x 36 cm plastic trays and labeled properly. The trays were kept at a temperature of 26-30 ºC and a relative humidity of 76% ± 5. They were being fed a diet consisting of ground dog biscuits or fish food. After the larvae has completed their metamorphosis into pupae, they were moved

into the net cages. Upon emergence of adults, they were kept at 25°C (±2) with a light/dark photoperiod cycle 12:12 and fed with 10% sugar solution soaked in cotton wool as shown in Fig. 2 (19). Susceptibility testing was performed on only female mosquitoes between the ages of 2 to 5 days (20). The adult Cx. quinquefasciatus were identified by using standard taxonomic keys under a stereomicroscope at 100X magnification (21, 22).

DELTAMETHRIN TREATED ITNS

By following the protocol and recommendation established by the World Health Organization, the efficacy of DawaPlusR 2.0 ITNs was evaluated (23). For this purpose, DawaPlusR 2.0 ITNs used in this research and were purchased from the local supplier. Polyester material impregnated exclusively with deltamethrin (2.7 g/kg) was used in the production of these nets (TANA Netting Company).

WHO TUNNEL BIOASSAY

The tunnel test was carried out according to the guidelines of WHO, to evaluate the behavioral reaction of mosquitoes towards insecticides (24). In this bioassay, the non-blood fed and non-sugar fed for 7-8 hours' female mosquitoes were used.

EVALUATION OF INSECTICIDE-TREATED NETS (ITNS) AGAINST CX. QUINQUEFASCIATUS

In order to evaluate the DawaPlusR 2.0 deltamethrin treated bed nets, a glass cylinder with dimensions of 25cm (height), 25cm (width) and 60cm (length) was used as shown in Fig. 3. The glass cylinder was divided into two sections by placing a piece of ITN between them. The piece of ITN had 9 holes, each measuring 1cm in diameter, to evaluate the efficacy of ITN against mosquitoes' mortality and feeding behavior. At dusk, a hen was placed in one chamber, which act as an animal bait and about 100 female mosquitoes were released into the other chamber. The glass cylinder was covered with a black cloth for whole night. Mosquitoes were aspirated out from the tunnel the next morning, during the time frame of 7:00 to 9:00 am. Similar procedure was adopted for control group except that the net was not treated with the insecticide. The number of surviving and dead mosquitoes, along with their fed or unfed condition, ascertain through abdominal observations, were documented from every chamber.

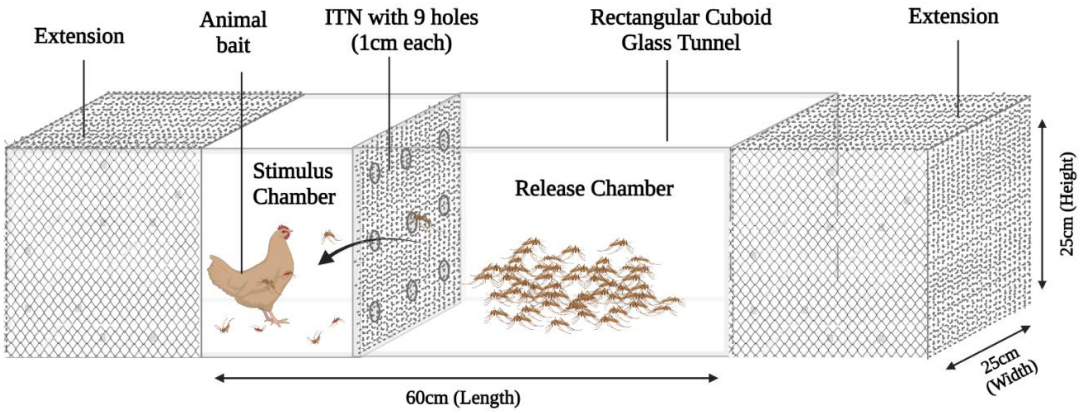

Fig. 3. The process of WHO Tunnel Bioassay explained in a photographic way.

DATA ANALYSIS

For this purpose, statistical software SPSS Statistics 26 was used. The percentage mortality, passage and blood-feeding were calculated and compared with the control group. The Chi-square (X2) test was performed to compare the variables and control groups. A P-value <0.05 was considered significant.

RESULTS

EFFICACY OF DELTAMETHRIN-TREATED NET (2.7G/KG) AGAINST CX. QUINQUEFASCIATUS MOSQUITOES

The mortality rate was highest in N-4 70.78% (109/154), followed by N-2 66.89% (101/151), N-3 62.68% (89/142), and N-1 50.80% (95/187), from the tehsil Narowal (district Narowal). From the tehsil Zafarwal (district Narowal), the highest mortality rate was recorded from N-7 77.07% (121/157), followed by N-6 72.22% (117/162), N-5 61.93% (109/176) and N-8 61.54% (104/169), respectively. From the tehsil Gujrat (district Gujrat), the rate of mortality was highest from G-3 71.52% (113/158), followed by G-2 69.48% (107/154), G-4 54.19% (97/179) and G-1 43.37% (121/279), respectively. While from the tehsil Kharian (district Gujrat), the highest mortality rate was 53.85% from both G-7 (91/169) and G-8 (77/143), followed by G-5 51.09% (94/184) and G- 6 49.77% (108/217) (Table II).

Table II. The tunnel bioassay results showing the % mortality of Cx. quinquefasciatus against deltamethrin treated net (2.7g/kg)

Tehsil	Collection Site	% Mortality		X^2 Value	p-value
		Control	ITN		
Narowal	N-1	3.85 (3/78)	50.80 (95/187)	1.49	~0.2222
	N-2	4.17 (3/72)	66.89 (101/151)	4.93	~0.0262*
	N-3	3.45 (2/58)	62.68 (89/142)	0.21	~0.6447
	N-4	3.13 (2/64)	70.78 (109/154)	0.04	~0.8355
Zafarwal	N-5	3.70 (3/81)	61.93 (109/176)	1.65	~0.1982
	N-6	2.90 (2/69)	72.22 (117/162)	4.57	~0.0326
	N-7	4.05 (3/74)	77.07 (121/157)	2.11	~0.1467
	N-8	2.04 (1/49)	61.54 (104/169)	1.89	~0.1699
Gujrat	G-1	3.06 (3/98)	43.37 (121/279)	4.68	~0.0307
	G-2	2.74 (2/73)	69.48 (107/154)	0.60	~0.4386
	G-3	0.00 (0/47)	71.52 (113/158)	2.89	~0.0883
	G-4	3.70 (3/81)	54.19 (97/179)	0.70	~0.4012
Kharian	G-5	2.63 (2/76)	51.09 (94/184)	0.46	~0.4995
	G-6	1.23 (1/81)	49.77 (108/217)	1.53	~0.2165
	G-7	2.98 (2/69)	53.85 (91/169)	1.85	~0.1738
	G-8	3.41 (3/88)	53.85 (77/143)	0.33	~0.5644

*Among the collection sites, only the collection site N-2 has a significant association with mortality ($p \approx 0.0262$)
Other collection sites do not show a significant association with mortality ($p > 0.05$)

The highest passage of Cx. quinquefasciatus through deltamethrin treated net was observed from tehsil Narowal (district Narowal) in N-3 57.04% (81/142), followed by N-1 49.73% (93/187), N-2 45.03% (68/151), and N-4 42.21% (65/154). Similarly, from tehsil Zafarwal (district Narowal), the highest rate of passage of Cx. quinquefasciatus across deltamethrin treated net was observed from N-6 58.02 (94/162), followed by N-8 57.99% (98/169), N-7 49.68% (78/157), and N-5 42.61% (75/176). From tehsil Gujrat (district Gujrat), the highest passage rate of Cx. quinquefasciatus through deltamethrin treated net was observed from G-1 65.59% (183/279), which was followed by G-2 56.49% (87/154), G-3 50.00 (79/158) and G-4 39.66% (71/179). Furthermore, from tehsil Kharian (district Gujrat), the highest rate of passage of Cx. quinquefasciatus across deltamethrin treated net was observed from G-8 31.47% (45/143), followed by G-7 20.71% (35/169), G-6 19.82% (43/217), and G-5 15.76% (29/184) (Table III).

From tehsil Narowal (district Narowal), the highest percentage of blood-feeding was observed in mosquitoes collected from N-3 40.85% (58/142), followed by N-4 30.52% (47/154), N-2 28.48% (43/151), and N-1 27.27% (51/187). Similarly, from tehsil Zafarwal (district Narowal), N-8 48.52% (82/169), had the highest rate of Cx. quinquefasciatus blood feeding, which was followed by N-6 43.21% (70/162), N-7 40.76% (64/157), and N-5 32.95% (58/176). From tehsil Gujrat (district Gujrat), the rate of the blood feeding in Cx. quinquefasciatus was highest from G-1 56.63% (158/279), which was followed by G-2 50.65% (78/154), G-3 40.51% (64/158), and G-4 30.17% (54/179). Similarly, from tehsil Kharian (district Gujrat) highest rate of blood feeding was observed in Cx. quinquefasciatus collected from G-8 21.68% (31/143), which was followed by G-7 17.75% (30/169), G-6 15.69% (34/217), and G-5 10.33% (19/184) (Table IV).

Table III. The tunnel bioassay results showing the % passage of Cx. quinquefasciatus against deltamethrin treated net (2.7g/kg)

Tehsil	Collection Site	% Passage		X^2 value	P-value
		Control	ITN		
Narowal	N-1	78.21 (61/78)	49.73 (93/187)	13.10	~0.0003
	N-2	58.33 (42/72)	45.03 (68/151)	13.71	~0.0002
	N-3	62.07 (36/58)	57.04 (81/142)	0.62	~0.4316
	N-4	64.06 (41/64)	42.21 (65/154)	9.68	~0.0019
Zafarwal	N-5	59.26 (48/81)	42.61 (75/176)	0.00	~0.9961
	N-6	71.01 (49/69)	58.02 (94/162)	2.47	~0.1159
	N-7	55.41 (41/74)	49.68 (78/157)	0.82	~0.3641
	N-8	77.55 (38/49)	57.99 (98/169)	3.58	~0.0580
Gujrat	G-1	72.45 (71/98)	65.59 (183/279)	4.62	~0.0316
	G-2	79.45 (58/73)	56.49 (87/154)	10.85	~0.0009
	G-3	65.96 (31/47)	50.00 (79/158)	9.78	~0.0018
	G-4	59.26 (48/81)	39.66 (71/179)	4.92	~0.0264
Kharian	G-5	48.68 (37/76)	15.76 (29/184)	7.17	~0.0074
	G-6	60.49 (49/81)	19.82 (43/217)	20.54	~0.0000
	G-7	59.42 (41/69)	20.71 (35/169)	6.06	~0.0141
	G-8	57.95 (51/88)	31.47 (45/143)	0.02	~0.8859

*Among the collection sites, only the collection site N-1, N-2, N-4, N-6, G-1, G-2, G-3, G-5, and G-6 has a significant association with passage. Other collection sites do not show a significant association with passage ($p > 0.05$)

Table IV. The tunnel bioassay results showing the % blood-feeding of Cx. quinquefasciatus against deltamethrin treated net (2.7g/kg)

Tehsil	Collection Site	% Blood-feeding		X^2 value	P-value
		Control	ITN		
Narowal	N-1	43.59 (34/78)	27.27 (51/187)	1.08	~0.2985
	N-2	26.39 (19/72)	28.48 (43/151)	15.43	~0.0001
	N-3	48.28 (28/58)	40.85 (58/142)	0.51	~0.4754
	N-4	48.44 (31/64)	30.52 (47/154)	5.60	~0.0178
Zafarwal	N-5	45.68 (37/81)	32.95 (58/176)	1.22	~0.2692
	N-6	53.62 (37/69)	43.21 (70/162)	0.11	~0.7420
	N-7	54.05 (40/74)	40.76 (64/157)	0.03	~0.8603
	N-8	67.35 (33/49)	48.52 (82/169)	2.87	~0.0890
Gujrat	G-1	58.16 (57/98)	56.63 (158/279)	1.84	~0.1755
	G-2	69.86 (51/73)	50.65 (78/154)	5.89	~0.0152
	G-3	76.60 (36/47)	40.51 (64/158)	3.01	~0.0834
	G-4	50.62 (41/81)	30.17 (54/179)	3.11	~0.0772
Kharian	G-5	40.79 (31/76)	10.33 (19/184)	6.22	~0.0126
	G-6	39.51 (32/81)	15.69 (34/217)	9.90	~0.0016
	G-7	59.42 (41/69)	17.75 (30/169)	0.04	~0.8459
	G-8	38.64 (34/88)	21.68 (31/143)	0.02	~0.8891

*Among the collection sites, only the collection site N-2, N-4, G-2, G-5, and G-6 has a significant association with blood-feeding. Other collection sites do not show a significant association with passage ($p > 0.05$)

DISCUSSION

In the presence of indoor vector control instruments, mosquitoes host seeking behavior specifically physiologically resistant mosquitoes must be monitored to assess if the tools efficiency is effective or need to be optimized (25). According to our findings, overall mortality was highest in Narowal tehsil, whereas passing rate were highest in Zafarwal tehsil. In comparison to the other tehsils, Gujrat tehsil has significantly lower mortality rates but significantly higher blood-feeding percentages. The Kharian tehsil demonstrated the lowest passage and blood feeding rates. It appears that the effectiveness of deltamethrin-treated nets upon Cx. quinquefasciatus mosquitoes varies across tehsils and collecting sites, even when using the same dose of the insecticide. Philip Oladele and his colleagues (2022) found 72% mortality rate in Culex species when treated with deltamethrin, indicating 28% mosquitoes exhibit resistance to the insecticide (26). The result of our findings aligns with this finding, indicating mortality rate ranged between 50.80 % to 77.07 % across different sites, though the mortality rate increased as compared to the control group, but it does not give 100% result, showing presence of resistant mosquitoes. However, this finding contradicts the result

reported by Tahir *et al.* (2009), who observed that by application of deltamethrin in accordance with WHO guideline let to a 100% susceptibility (27).

We also evaluate the percentage of mosquitoes that were able to pass through the net and take blood feed. In contrast to control group there was subsequent decrease in penetration and resulting in a reduced number of mosquitoes able to successfully obtain a blood meal. This reduction basically revealed the information about the insecticide's repelling impact and the degree to which that effect was connected with mortality. Similar studies were done by Rafinejad *et al.,* (2008) where reduction in penetration of mosquitoes was observed through ITNs (28). In another study done by Richard M. Oxborough *et al.,* (2013), where in control group the majority of mosquitoes penetrate (87%) and eventually engaged in blood feed (81%) while in insecticide treated group there was considerable decrease in both penetration (52% to 60%) and blood feed (38% to 41%) (29). The aforementioned findings suggest that the efficacy of deltamethrin-treated nets can be influenced by geographical factors, mosquito behavior and patterns of resistance. Additional research is necessary causes for these differences and to explore supplementary approaches for managing populations of Cx. quinquefasciatus in diverse geographical regions. In order to comprehend the underlying causes where ITNs failed to provide protection against resistant mosquitoes, it is essential to conduct an examination of the various factors and determinants that contribute to the ineffectiveness of the ITNs. To achieve this goal, investigating many types of resistance, including metabolic resistance and resistance at the intended target site is required.

CONCLUSION

The research findings indicate that the implementation of deltamethrin-treated nets containing a concentration of 2.7g/kg exhibits the capacity to effectively decrease populations of Cx. quinquefasciatus mosquitoes. However, the effectiveness of the nets varies greatly depending on their collection site. The findings of this study emphasize the significance of taking into consideration the contextual variables, when implementing interventions for mosquito control. Additional investigation and surveillance are required in order to formulate precise and efficient approaches for the management of Cx. quinquefasciatus in particular geographical areas including metabolic and target site resistance.

Acknowledgments:

The images were created by using Biorender.com and mapping is done by ArcGIS software.

Conflicts of Interest:

The authors declare that they have no conflicts of interest.

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