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**Ripu Daman Sood**

(a) National Institute of Malaria  
Research, Sector 8 Dwarka, New Delhi  
(b) Indira Gandhi National Open  
University, Maidan Garhi, New  
Delhi.

**Neera Kapoor**

Indira Gandhi National Open  
University, Maidan Garhi, New  
Delhi, India.

**P.K.Mittal**

National Institute of Malaria  
Research, Sector-8, Dwarka, New  
Delhi, India.

# A comparative study on bio-efficacy and wash resistance of five long lasting insecticidal mosquito nets against malaria vector *Anopheles culicifacies*

**Ripu Daman Sood, Neera Kapoor, P.K. Mittal**

## ABSTRACT

The study was carried out to test the wash resistance and bio-efficacy of five long lasting insecticidal nets (LLINs) namely Olyset® Net, PermaNet® 2.0, Icon Life®, Duranet®, and Interceptor® net against *Anopheles culicifacies*, a major rural malaria vector in India. Mortality and median knockdown time (MKDT) of mosquitoes on five different LLINs, fresh and washed up to 20 times, were determined by using cone bioassays and ring net bioassays, respectively. LLINs showed high efficacy against *An. culicifacies*. The mortality of mosquitoes remained >80% even after 20 hand washes on all the five LLINs tested. There was a significant difference in MKDT of *An. culicifacies* on different LLINs. MKDT increased progressively with successive washes. The study revealed that all the LLINs were effective to produce >80% mortality against *An. culicifacies* even after 20 washes. However, there was a significant difference in MKDT between fresh nets and 20 times washed nets in case of all the five LLINs. After 20 washes, the lowest MKDT was observed on PermaNet® 2.0, while the highest MKDT was observed on Interceptor® net.

**Keywords:** Efficacy, LLIN, *An. culicifacies*, malaria vector

## 1. Introduction

*Anopheles culicifacies* is one of the major malaria vectors in India and it transmits 60-70% of all malaria cases in India [1]. So far the major strategy for the control of *An. culicifacies* in India is based mainly on indoor residual spraying. Control of malaria vectors in India now takes a shift from indoor residual spraying to the Insecticide treated mosquito nets (ITNs) in high risk areas. In recent years long lasting insecticidal mosquito nets (LLINs) are being promoted for the control of malaria vectors. Development of this technology has provided a solution for the variety of problems related to the ITNs such as dipping, spraying, erratic dose of insecticide over the fibers, and loss of insecticide due to washing etc. LLINs are playing an important role in the vector control due to their sustaining strength against malaria vectors. These nets not only provide personal protection from the mosquitoes but also reduce the transmission of malaria. At present there are only 3 LLIN viz., Olyset® net, PermaNet® 2.0 and Yorkool® net, which have got full recommendations from World Health Organization Pesticide Evaluation Scheme (WHOPES) for use in vector control, while other LLIN including Icon life® (Netprotect®), Duranet® and Interceptor® net have got only interim recommendations. (www.who.int/whopes) [2]. Wash resistance and bioefficacy of Olyset® net and PermaNet® 2.0 against malaria vectors have been studied thoroughly by various researchers in different countries, including India [3-13]. But there are fewer reports on other LLINs. Icon Life® (also marked as Netprotect®) a deltamethrin incorporated polyethylene net developed by the M/s Syngenta Ltd., and Duranet® incorporated with alphacypermethrin developed by Clarke Mosquito control have been reported to show varying degree of efficacy against malaria [14-16]. Interceptor net manufactured by BASF (Germany) is an alphacypermethrin coated long-lasting insecticidal mosquito net with the target dose of 200 mg of alphacypermethrin per square metre of the polyester fabric. It is one of the interim recommended long lasting insecticidal net approved by the WHOPES [17].

In the present study, the bio-efficacy and wash resistance of five LLINs viz., Olyset® PermaNet® 2.0, Icon Life®, Duranet® and Interceptor® net before and after different washings against *An. culicifacies*, a major rural vector of malaria in India has been compared.

**Correspondence:**

**Dr. P.K. Mittal**

National Institute of Malaria  
Research, Sector 8, Dwarka, New  
Delhi, India.

Email: pk\_mittal52@yahoo.co.in

## 2. Materials and Methods

### 2.1 Test mosquitoes

Blood fed female *An. culicifacies* mosquitoes were collected from houses and cattle sheds in villages of Distt. Gautam. Budh Nagar U.P. and kept in mosquito cages in the laboratory. *An. culicifacies* were separated and categorized on the basis of their abdominal condition as gravid, semi gravid and full fed. Gravid *An. culicifacies* collected from field mosquitoes were kept in cloth cages and allowed to lay eggs on water taken in plastic bowls. These eggs were reared to obtain fl generation in the laboratory for cone and ring net bioassays.

### 2.2 LLIN Products

In the present study, five LLIN products viz. Olyset<sup>®</sup>, PermaNet<sup>®</sup> 2.0, Icon Life<sup>®</sup> (Netprotect<sup>®</sup>), Duranet<sup>®</sup> and Interceptor<sup>®</sup> were used for comparative study on bioefficacy and wash resistance in the laboratory. Untreated nets were used as control.

### 2.3 Olyset<sup>®</sup>

Olyset<sup>®</sup> is a long lasting insecticidal net incorporated with permethrin 2% at factory level manufactured by M/s Sumitomo Chemicals, Japan. Olyset<sup>®</sup> was provided gratis by M/s Sumitomo Chemicals India Pvt. Ltd., Mumbai, India. Polyethylene untreated nets were used for comparison with same hole size and thickness which were also supplied by M/s Sumitomo Chemicals India Pvt. Ltd., Mumbai, India.

### 2.4 PermaNet<sup>®</sup> 2.0

PermaNet<sup>®</sup> 2.0, manufactured by M/s Vestergaard Frandsen of Denmark, is a polyester net coated with deltamethrin @ 55 mg/ m<sup>2</sup>. These nets were provided gratis by M/s Vestergaard Frandsen India Pvt. Ltd., New Delhi, India.

### 2.5 Icon Life<sup>®</sup> net (Netprotect<sup>®</sup>)

It was marketed under two trade names, Icon Life<sup>®</sup> (distributed by Syngenta and Net protect distributed by Best net Europe Ltd<sup>14</sup>. Icon Life<sup>®</sup> is incorporated polyethylene net containing 65 mg.a.i./m<sup>2</sup> of deltamethrin on its fibers.

### 2.6 Dura net<sup>®</sup>

Duranet<sup>®</sup> is a LLIN product incorporated with alphacypermethrin into polyethylene fibers developed by the Clarke Mosquito Control USA. It contains 0.55%ww ± 15% alphacypermethrin.

### 2.7 Interceptor<sup>®</sup> nets

Interceptor<sup>®</sup> net manufactured by BASF (Germany) is an alphacypermethrin long-lasting insecticidal polyester net coated with the target dose of 200 mg of alphacypermethrin per square meter.

### 2.8 Test procedures

Cone and ring net bioassays were performed to test the efficacy of nets in producing mortality and knockdown.

Cone bioassay tests on LLIN were performed as per WHO standard procedures <sup>[18]</sup>. Plastic cones were used for these bioassays. Net samples of 25 cm x 25 cm were fixed to a cardboard and the cone was placed above the net and fixed with tape. Cones were affixed vertically on the netting to avoid the resting of mosquitoes on the

surface of the cones. Five susceptible 3-5 day old non blood fed (glucose fed) *An. culicifacies* mosquitoes were exposed to netting materials for 3 minutes. After 3 minutes exposure, mosquitoes were removed from cone using the suction tube and put in paper cup covered with an untreated plain net. The mosquitoes were provided with cotton soaked in glucose solution and placed on the untreated netting covering. After that, these paper cups were kept in an environmental chamber maintained at 27 ± 2 °C and 80 % RH and knockdown was scored after 1 hour and mortality after 24 hours, respectively. Cone bioassays were performed in four replicates for each species. The untreated nets were taken as control. All data were pooled together.

Ring net bioassays were conducted following the guidelines given by WHO <sup>[19]</sup>. A netting apparatus consisting of two intersecting circles of 15 cm diameter; welded together to get sphere shape, wrapped with the net sample (25cm x 25 cm) was used for ring net bioassays. Eleven mosquitoes were introduced and the time required for the knockdown of 1<sup>st</sup>, 6<sup>th</sup>, and 11<sup>th</sup> mosquito was recorded. The time required for the knock-down of 6<sup>th</sup> mosquito was taken as the median knockdown time (MKDT). This was done in 4 replicates.

### 2.9 Net washing

To assess wash resistance of the LLIN, one piece each measuring 25 x 25 cm was cut from four nets and washed with a commercial detergent, Surf Excel (Hindustan Unilever Private Ltd., Mumbai, India). One teaspoon (5 g) of the detergent was dissolved in 5 liters of water and net pieces were dipped in the detergent solution for 10 min. The pH of the detergent solution was 9.0 to 9.5. Subsequently, the net pieces were thoroughly rinsed with tap water and dried under shade for 8 h. After drying, these nets were packed in a polyethylene bag and stored in a cupboard at room temperature until the next wash. All the nets were washed at 7-day intervals. These nets were washed 20 times using the same procedure and after every 5,10,15,20 washes, pair of 25 cm x 25 cm samples were stored after drying in shade. After drying, these nets were packed in a polyethylene bag and kept in dark and cool place at ambient temperature in a cupboard until the next wash. Bioassays were conducted on day 1, the next day after 5<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup>, 20<sup>th</sup> wash against all the LLINs.

### 2.10 Data analysis

All data were subjected to statistical analysis using MS-excel, epiinfo Open source epidemiologic statistics for public health, version 2.3.1. (centre for disease control and prevention, Atlanta, GA) software. Comparison of the efficacy and wash resistance of the five LLINs was made using Student t-test and analysis of variance.

## 3. Results

Comparative bio-efficacy and wash resistance of five long lasting insecticidal net products was assessed by cone bioassays and ring net bioassays in the laboratory. Wash resistance of LLINs were tested by determining mortality and MKDT after 5,10,15 and 20 consecutive washes using two different bioassays.

**Table 1:** Per cent mortality of *An. culicifacies* exposed on fresh unwashed Olyset<sup>®</sup>, PermaNet<sup>®</sup>2.0, Icon Life<sup>®</sup>, Duranet<sup>®</sup> and Interceptor<sup>®</sup> nets in WHO cone bioassays

LLIN	<i>An. culicifacies</i>	
	Knockdown after 1 hr	Mortality after 24 hrs
Olyset <sup>®</sup> net	5±0 (100 %)	5±0 (100 %)
PermaNet <sup>®</sup> 2.0	5±0 (100 %)	5±0 (100 %)
Icon Life <sup>®</sup> net	4.8±0.63 (96%)	5±0 (100 %)
Interceptor <sup>®</sup> net	4.8±0.42 (96%)	5±0 (100 %)
Duranet <sup>®</sup>	5±0 (100 %)	5±0 (100 %)
F value	1.046	-
P value	P=0.3939	-

\* Total 10 replicates were used and 5 mosquitoes were tested against each net. Data were pooled for each type of net.

Table 1 shows per cent mortality of *An. culicifacies* on five LLINs in cone bioassays. All five unwashed LLINs produced 100% mortality after 24 hours in comparison to untreated netting. Per cent knock down after 1 hour was 96% for Icon Life<sup>®</sup> and Interceptor<sup>®</sup> nets and 100% for Olyset<sup>®</sup>, PermaNet<sup>®</sup> and Duranet<sup>®</sup>. Though

different LLINs showed some variation in the knock down percentage after 1 hour, no difference was observed in mortality after 24 hours. Untreated control net did not produce any mortality of *An. culicifacies*.

**Table 2:** MKDT (in minutes) of *An. culicifacies* exposed to fresh unwashed Olyset<sup>®</sup>, PermaNet<sup>®</sup>, Icon Life<sup>®</sup>, Duranet<sup>®</sup> and Interceptor<sup>®</sup> nets as determined by ring net bioassays.

LLIN	<i>An. culicifacies</i>		
	1 <sup>st</sup> mosquito	6 <sup>th</sup> mosquito	11 <sup>th</sup> mosquito
Olyset <sup>®</sup> net	3.8 ±0.32	<b>5.0±0.33<sup>d</sup></b>	7.25± 0.26
PermaNet <sup>®</sup> 2.0	3.6±0.52	<b>5.2±0.25<sup>d</sup></b>	7.5±0.52 <sup>e</sup>
Icon Life <sup>®</sup> net	3.4±0.51	<b>5.5±0.40<sup>d</sup></b>	7.8±0.34
Interceptor <sup>®</sup> net	3.2±0.63	<b>8.0±0.43<sup>abce</sup></b>	10.0±0.52
Duranet <sup>®</sup>	3.1±0.61	<b>5.0±0.40<sup>d</sup></b>	7.8±0.52
F value	2.9246	<b>133.0</b>	61.19
P value	0.03*	<b>&lt;0.0001*</b>	<0.0001*

\*Total 10 replicates were used and 11 mosquitoes were tested against each net. Data were pooled for each type of net.

Olyset<sup>®</sup>, PermaNet<sup>®</sup>2.0, Icon Life<sup>®</sup>, Interceptor<sup>®</sup> and Duranet<sup>®</sup> are symbolized as superscript <sup>a,b,c,d,e</sup> respectively Value in a corresponding cell is significantly different from the values of the superscripts. Student t test was used to compare individual LLINs. Analysis of variance was performed to test the statistical significance. Asterisk indicates significant difference (P < 0.05).

Table 2 shows the MKDT, (time required for the knockdown of 6<sup>th</sup> mosquito) for *An. culicifacies* on five LLINs in ring net bioassays. MKDT for *An. culicifacies* was 8.0 ±0.43 minutes on Interceptor<sup>®</sup>

net and was significantly different from the other LLINs (P<0.0001).

**Table 3:** No dead/No tested\* from fresh unwashed and washed Olyset<sup>®</sup>, PermaNet<sup>®</sup>2.0, Icon Life<sup>®</sup>, Duranet<sup>®</sup> and Interceptor<sup>®</sup> nets bioassayed with Three minutes exposure + 24 h holding of *An.culicifacies* in WHO cones.

Status of washing	Olyset <sup>®</sup> net	PermaNet <sup>®</sup> 2.0	Icon Life <sup>®</sup> net	Interceptor <sup>®</sup> net	Duranet <sup>®</sup> net	F value	P value
Fresh (unwashed net)	5±0 (100 %)	5±0 (100 %)	5±0 (100 %)	5±0 (100 %)	5±0 (100 %)	-	-
5 time washed net	5±0 (100 %)	5±0 (100 %)	5±0 (100 %)	5±0 (100 %)	5±0 (100 %)	-	-
10 time washed net	5±0 (100 %)	5±0 (100 %)	4.5±0.57 (90%)	4.5±0.57 (90%)	4.75±0.5 (95%)	1.38	0.28
15 time washed net	4.75±0.5 (95%)	4.75±0.5 (95%)	4.25±0.5 (85%)	4.25±0.5 (85%)	4.5±0.57 (90%)	0.94	0.46
20 time washed net	4.5±0.57 (90%)	4.25±0.5 (85%)	4±0.81 (80%)	4±0.81 (80%)	4.25±0.5 (85%)	0.48	074

\* Total 4 replicates were used and 5 mosquitoes were tested against each net. (5 x 4 =20) .Data were pooled for each type of net.

Table 3 shows per cent mortality of *An. culicifacies* in cone bioassays on five LLINs after different number of washings. All five LLINs showed 100% mortality on fresh nets, but as the

number of washes increased, per cent mortality declined. There was significant difference in the per cent mortality of *An. culicifacies* between fresh unwashed and 20 times washed LLINs

in case of permaNet<sup>®</sup>2.0, Icon Life<sup>®</sup> and Interceptor<sup>®</sup> net ( $p < 0.05$ ), but no significant difference was observed between fresh unwashed and 20 times washed Olyset<sup>®</sup> net and Duranet<sup>®</sup> ( $P > 0.05$ ). However, all five LNs showed more than 80% mortality against *An. culicifacies* even after 20 washes in cone bioassays (Table 3).

Results of cone bioassays with *An. culicifacies* revealed  $>95\%$  knockdown after 1 hour and  $\geq 80\%$  mortality after 24 hours on all five insecticidal net products even after 20 washes, hence all these mosquito nets fulfilled the criteria to be a LLIN as laid down by WHO.

**Table 4:** Mean  $\pm$  S.D of MKDT (in minutes) of *An. culicifacies* exposed to fresh unwashed and washed Olyset<sup>®</sup>, PermaNet<sup>®</sup>2.0, Icon Life<sup>®</sup>, Duranet<sup>®</sup> and Interceptor<sup>®</sup> nets

Status of washing	Olyset <sup>®</sup> net	PermaNet <sup>®</sup> 2.0	Icon Life <sup>®</sup> net	Interceptor <sup>®</sup> net	Duranet <sup>®</sup> net	F value
Fresh net (unwashed net)	5.0 $\pm$ 0.40 <sup>d</sup>	5 $\pm$ 0.57 <sup>d</sup>	5.0 $\pm$ 0.40 <sup>d</sup>	8 $\pm$ 0.40 <sup>abce</sup>	5 $\pm$ 0.70 <sup>d</sup>	27.801
5 time washed net	7.2 $\pm$ 0.47 <sup>d</sup>	6.2 $\pm$ 0.67 <sup>cde</sup>	7.5 $\pm$ 0.70 <sup>bd</sup>	10 $\pm$ 0.70 <sup>abce</sup>	7.5 $\pm$ 0.81 <sup>bd</sup>	17.061
10 time washed net	8.5 $\pm$ 0.40 <sup>bd</sup>	6.8 $\pm$ 0.95 <sup>ace</sup>	8.6 $\pm$ 0.57 <sup>bd</sup>	12 $\pm$ 0.40 <sup>abce</sup>	8.5 $\pm$ 0.57 <sup>bd</sup>	38.530
15 time washed net	9.2 $\pm$ 0.24 <sup>bcd</sup>	7.8 $\pm$ 0.54 <sup>acde</sup>	9.75 $\pm$ 0.28 <sup>abde</sup>	14 $\pm$ 0.43 <sup>abce</sup>	9.0 $\pm$ 0.41 <sup>bcd</sup>	144.312
20 time washed net	9.6 $\pm$ 0.86 <sup>d</sup>	9 $\pm$ 0.54 <sup>d</sup>	10 $\pm$ 0.91 <sup>d</sup>	16.5 $\pm$ 0.70 <sup>abce</sup>	9.5 $\pm$ 0.21 <sup>d</sup>	82.368
Regression equation	Yt=5.66+0.224Xt	Yt=5.04+0.192Xt	Yt=5.72+0.245Xt	Yt=7.9+0.42Xt	Yt=5.8+0.21Xt	

\* Total 4 replicates were used and 11 mosquitoes were tested against each net (11 x 4=44 mosquitoes). Data were pooled for each type of net. Olyset<sup>®</sup>, PermaNet<sup>®</sup>, Icon Life<sup>®</sup>, Interceptor<sup>®</sup> and Duranet<sup>®</sup> are symbolized as superscript <sup>a,b,c,d,e</sup> respectively. Value in a corresponding cell is significantly different from the values of the superscripts. Student t test was used to compare individual LLINs. Analysis of variance was performed to test the statistical significance. Asterisk indicates significant difference ( $P < 0.05$ )

Table 4 shows MKDT of *An. culicifacies* on five LLIN products, after different number of washings in Ring-net bioassays. MKDT for all the LLINs increased from unwashed nets to 20 times washed nets. There was a significant difference in MKDT between fresh nets and 20 times washed nets in case of all the five LLINs ( $P < 0.05$ ). After 20 washes, the lowest MKDT (9  $\pm$  0.54) was observed on PermaNet<sup>®</sup>2.0 as compared to (16.5  $\pm$  0.70) on Interceptor<sup>®</sup> net. In case of Olyset<sup>®</sup> nets, the MKDT increased from 5.0 to 9.6 minutes after 20 washes, while on PermaNet<sup>®</sup> 2.0 MKDT increased from 5 to 9 minutes. Icon Life<sup>®</sup> net showed an increase in MKDT from 5.0 to 10, whereas Duranet<sup>®</sup> showed an increase from 5.0 to 9.5 minutes. Interceptor<sup>®</sup> net showed the maximum increase in the MKDT from 8  $\pm$  0.40 to 16.5  $\pm$  0.70 after 20 washes.

#### 4. Discussion

From the present study, the efficacy of Olyset<sup>®</sup> net & PermaNet<sup>®</sup> 2.0, which were given full recommendation by WHO<sup>2</sup>, were compared along with Icon Life<sup>®</sup> net, Duranet<sup>®</sup> & Interceptor<sup>®</sup> net, which have been given only interim recommendations, to get a fair idea about the efficacy of these LLINs against *An. culicifacies*. The study revealed a high efficacy of all the LLINs against *An. culicifacies*, a rural vector of malaria in India. Mortality of mosquito species exceeded 80% on nets washed up to 20 times by hand. It may be because *An. culicifacies* used in these tests was highly susceptible to synthetic pyrethroids, which are used in LLINs. The efficacy lasted even after 20 washes which proved wash resistance of these LLINs. The amount of insecticide left over on the nets might have been sufficient to produce  $>80\%$  mortality in these mosquitoes as evident in cone bioassay tests. The study also observed that all the five brand of LLINs evaluated, were

equally effective against the vector species tested up to 10 washes, but after that each LLIN gave different results in terms of percent mortality. In the present study, Olyset<sup>®</sup> net showed highest per cent mortality (95%) against *An. culicifacies* after 20 washes which may be due to high insecticide content of the permethrin. These results are in confirmation with those of earlier reports on Olyset<sup>®</sup> net [8,9]. Sharma *et al.* [9] evaluated for the wash resistance and bioefficacy of Olyset net against *An. culicifacies* and *An. fluviatilis* in India and showed 100% mortality in *An. fluviatilis* after 20 washes, where as in *An. culicifacies* 100% mortality was seen up to 11 washes, which declined to 80% after 20 washes. The density of both the vectors was significantly reduced in houses with Olyset nets compared with untreated nets or no nets. Ansari *et al.* [8], reported 90% mortality against the *An. culicifacies* even after 20 washes. However, another study carried out by Rafineejad *et al.* [20] reported only 9% mortality against *An. stephensi* after 20 washes. These variations in the per cent mortality of anophelines on washed LLINs may be due to different susceptibility status and species differences.

In the present study, PermaNet<sup>®</sup> 2.0 recorded 85% mortality against *An. culicifacies* after 20 washes. Similarly  $>80\%$  mortality in *Anopheles* mosquitoes on PermaNet<sup>®</sup> 2.0 after 21 to 23 washes have been reported by others [3, 6]. However, Prakash *et al.* [21] contradicts the wash resistance findings in which PermaNet<sup>®</sup> 2.0 showed 47.6% mortality after 16-22 washes against *An. minimus*. Jaramillo *et al.* [22] also reported the reduced performance of PermaNet<sup>®</sup> 2.0 as it showed only 60% mortality against *An. albimanus* after 20 washes under laboratory conditions. In the present study, Icon Life<sup>®</sup> (Net protect<sup>®</sup>) (incorporated with deltamethrin at the dose of 65mg/m<sup>2</sup> on polyethylene) showed 80% mortality against *An. culicifacies* after 20 washes, whereas 100%

mortality of field collected *An. culicifacies* was recorded on Icon Life® LLIN even after 20 washes in an earlier study<sup>[16]</sup>. Dev *et al.*<sup>[15]</sup> showed >80% mortality on Net Protect® (Icon Life®) against *An. minimus* respectively. In another study mentioned by WHO report Netprotect® showed 76% mortality against *An. gambiae* after 20 washes under laboratory conditions<sup>[14]</sup>. In the present study, Interceptor® net (coated with alphacypermethrin at the dose of 200mg/m<sup>2</sup> on polyester) showed 80% mortality for *An. culicifacies* after 20 washes. Similar type of results have been reported earlier where Interceptor net showed 80% mortality against *An. gambiae* after 20 washes<sup>[17]</sup>. In the present study, Duranet® (incorporated with alphacypermethrin at the dose of 261mg/m<sup>2</sup> on polyethylene) showed 85% mortality for *An. culicifacies* after 20 washes. However Duranet® showed 100% knock-down but only 45% mortality after 20 washes against *An. Gambiae*<sup>[14]</sup>. These variations in the % mortality and MKDT of anophelines on washed LLINs may be due to different susceptibility status and species differences.

In the present study, all five insecticidal nets showed ≥80% mortality and ≥95% knock down after 1 hr against *An. culicifacies* even after 20 washes in cone bioassays. Hence each net satisfied the criteria to be LLIN<sup>[18]</sup>.

These finding suggests that cone bioassays alone are not adequate to assess the comparative efficacy of these nets, therefore more elaborate tests *viz.*, ring-net bioassay, which measure MKDT were performed to understand the bioavailability of the insecticide on the LLINs. MKDT, an important parameter, which is expected to be directly correlated to the insecticide concentration on the surface for fast acting pyrethroids, is helpful in assessing the amount of insecticide left on the net fibres<sup>[20]</sup>.

Comparison of MKDT values between LLINs showed PermaNet® to be most effective even after 20 washes, whereas Interceptor net was found to be least effective. Results of ring-net bioassays revealed an increased MKDT of *An. culicifacies* with increased number of washes in case of all the five LLINs. Of all the five LLINs, PermaNet® 2.0 showed highest wash resistance against *An. culicifacies*, which indicates less insecticide loss after every wash. MKDT of *An. culicifacies* on unwashed PermaNet® 2.0 was 5 minutes which increased to 9 minutes in 20 times washed LLIN. Similar type of results have been reported by the Gimnig *et al.*<sup>[5]</sup> in which MKDT increased with progressive number of washes. Gunasekaran and Vaidyanathan<sup>[12]</sup> also reported the better wash resistance in PermaNet 2.0 after successive number of washes. MKDT of *An. culicifacies* on Olyset® net after 20 washes was 9.6 minutes. In another study, Sreehari<sup>[13]</sup> reported that the MKDT of *An. culicifacies* on unwashed Olyset net increased from 311 seconds from unwashed nets to 457 seconds on 20 time washed nets and MKDT of *An. stephensi* on unwashed Olyset net increased from 349 seconds to 542 seconds in 20 times washed nets. In the present study, MKDT of *An. culicifacies* increased from 5 minutes on unwashed Icon Life® nets to 10 minutes on 20 times washed nets. Similar increase in MKDT from 354 seconds on unwashed Netprotect® nets to 1310 seconds on 20 times washed Netprotect® have been reported<sup>[14]</sup>.

Duranet® showed 5 minutes MKDT against *An. culicifacies* on unwashed nets, which increased to 9.5 minutes after 20 washes, while, Interceptor® net showed 8 minutes MKDT on unwashed nets which increased to 16.5 minutes after 20 washes against *An. culicifacies*.

Thus washing plays an important role in the bioavailability of the insecticide over the net fibres. All the LLINs showed an increase in MKDT, due to the loss of insecticide after washing, which was directly correlated with the increased number of washes. The efficacy of the LLIN is brand specific and has no relationship between efficacy and its manufacturing process whether coated or incorporated. Under same circumstances all the LLINs regenerate the insecticide accordingly. All the LLINs irrespective of their manufacturing differences showed >80% mortality in cone bioassays against *An. culicifacies*.

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