Field evaluation of permethrin long-lasting insecticide treated nets (Olyset®) for malaria control in an endemic area, southeast of Iran

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ARTICLE INFO

ABSTRACT

Long lasting insecticide treated nets (LLINs) have been advocated as an effective tool for prevention and control of malaria. Olyset net was the first LLINs which became commercially available and obtained WHO approval. According to the national strategic plan on evaluation of Olyset net, a field trial was conducted to determine the efficacy of these nets against malaria vectors in an endemic area in the southeast of Iran.

Fourteen villages with similar topographical and epidemiological situations were selected and randomly assigned to two clusters of the study: Olyset net and untreated net. Distribution of nets was carried out to cover 100% of the population in Olyset net and untreated net cluster.

Anopheline mosquitoes were collected monthly using different WHO standard methods in both areas to determine their abundance, feeding pattern and resting behaviour. Human blood index was determined using ELISA test. Additionally, Olyset nets were evaluated for their biological activity using WHO cone bioassay test by susceptible colony of *Anopheles stephensi* (Beech strain) and then for insecticide residues by employing high performance thin layer chromatography. Malaria incidence was measured by passive and active case detection from all study population.

In total 2115 adult anopheline mosquitoes were collected and identified using morphological characters. They comprised of seven species: *Anopheles dthali* (Liston), *A. culicifacies* (Giles), *A. stephensi* (Liston), *A. superpictus* (Grassi), *A. fluviatilis* (James), *A. moghulensis* (Christophers) and *A. turkhudi* (Liston). *A. dthali*, *A. culicifacies* and *A. stephensi* were most prevalent species in both areas.

In the Olyset net study area, there was a significant reduction of 41.1%, 54.4%, 59.39% and 64.1% in the indoor-resting density of *A. culicifacies*, *A. stephensi*, *A. dthali* and *A. superpictus*, respectively, with an overall reduction of 39.3% in total mosquitoes in comparison with untreated net area. A significant reduction was also observed in human blood index of vector species in the Olyset net villages. Bioefficacy test results of Olyset nets showed that the median knockdown time was 1.48 and 3.25 min, while the average mortality rate was 100% and $72.3\% \pm 7.07$ in baseline and after 1 year of intervention, respectively. The average permethrin content reached to 68.31% ($683.1 \, \text{mg/m}^2$) of the initial insecticide dose of $937 \pm 21.69 \, \text{mg/m}^2$ (nearly $1000 \, \text{mg/m}^2$) at the end of intervention.

Malaria incidence was reduced by 96.6% and 64.8% in the village with Olyset nets and in the villages with untreated nets, respectively. During intervention period, there was a reduction of 93.2% in malaria incidence in Olyset net area as compared to the untreated area.

This study indicated that Olyset nets have a major impact on malaria vectors and disease burden; therefore it could be recommended as an effective personal protection tool for malaria control in malarious areas.

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1. Introduction

Malaria is still a public health problem in southern part of Iran. According to the report of The Ministry of Health and Medical Education of the country there is a sharp decline of malaria trend since 15 years. Altogether a total of 33 *Anopheles*, including siblings, biological forms and genotypes were recorded. Among which 7 have been implicated as the main vector. In the southern part of the country there are six *Anopheles* mosquitoes including *A. stephensi* (Vatandoost et al., 2006), *A. culicifacies* (Emami et al., 2007), *A. dthali*, *A. fluviatilis* (Naddaf et al., 2003; Vatandoost et al., 2004), *A. superpictus* and *A. pulcherrimus* (Oshaghi et al., 2007). *A. sachacrovi* (Sedaghat et al., 2003a) and *A. maculipennis* (Oshaghi et al., 2003; Sedaghat et al., 2003b) are considered as malaria vector in northern part of the country. The main activities for vector control are indoor residual spraying using pyrethroid insecticides. Impregnated bed nets, larviciding using biological agents such as larvivorous, fishes and *Bacillus thuringiensis*, fogging and recently advocating and distribution of long-lasting insecticidal nets.

Long-lasting insecticidal nets (LLINs) are defined by WHO as nets treated with insecticide either incorporated into or coated around the fibres, which resist multiple washes and the biological activity lasts as long as the net itself (WHO, 2007).

Many countries in malarious area of Africa are currently scaling up the coverage of LLINs. These programs, funded by the Global Fund or President's Malaria Initiative, intend to have a lasting impact on malaria transmission (Breman, 2009). In 2008, 23 countries in the African region and 35 outside that region had adopted the WHO recommendation to provide bed nets for all age groups at risk for malaria; this represents an increase of 13 countries since 2007 (WHO, 2010).

These nets are designed to maintain their biological efficacy against vector mosquitoes for at least three years in the field under recommended conditions of use, obviating the need for regular insecticide treatment (WHO, 2009a, b). Out of the tree brands of LLIN currently approved by the WHOPES, Olyset® net (Sumitomo Chemical Co., Ltd., Japan) is the only one currently granted full recommendation (N'Guessan et al., 2001; Teklehaimanot et al., 2007). Olyset netting is made out of wide-meshed high-density polyethylene in which the insecticide (permethrin) is incorporated directly into the fibre at a 2% weight/weight concentration (corresponding to $1 g/m^2$ surface concentration). The extensive laboratory and field experience with Olyset nets are comprehensively reviewed in a WHOPES document (Lindblade et al., 2005; WHOPES, 2009).

Many studies in the malarious countries indicate that Olyset has good efficacy against different mosquito species and as a suitable personal protection tool (Ansari et al., 2006; Jeyalaksmi et al., 2006; Sharma et al., 2006; Sreehari et al., 2007).

The National Malaria Control Programmes in Iran currently rely on strategies targeting mosquito vector control, which involve the use of permethrin-impregnated Olyset net.

The free of charge distribution of Olyset nets initiated by the Centre for Diseases Management and Control (CDMC), Iran. In 2009, approximately 80,000 Olyset nets were distributed in malarious areas in south and southeast of the country (Iranian Ministry of Health, unpublished data).

Earlier study in Iran on Olyset nets revealed the effectiveness of these long-lasting nets against *A. stephensi* in laboratory condition (Rafinejad et al., 2008).

Field evaluation of Olyset nets is necessary for successful malaria control plan and no study has addressed this issue; therefore present study aimed to assess the efficacy of Olyset against malaria vectors in Bashagard, which is a malaria endemic focus in the southeast of Iran.

2. Materials and methods

2.1. Study area

The trial area, Bashagard district is situated in the Hormozgan province, southeast Iran. The district is located between 26°04′–26°58′ N latitude and 57°23′–59°02′ E longitude. The annual mean relative humidity is 40% and the average daily temperatures in the warmest and coolest months of the year are 44 °C and 9 °C, respectively. Entire district is 10,000 km² in area and has a population of 31,293. Approximately 90% of district's population live in rural area and the main economic activities are farming and livestock herding. Most of the region in Bashagard is mountainous with deep valley and steep slopes. The villages are small, scattered and relatively difficult to access with low population. Majority of the population live in hills and foot-hills and most of their houses are very low with thatched roofs. The sheds of domestic animal were built close to human dwelling and were generally made of straw or palm leaves. Malaria is a major public health problem in this district and occurs year-round with peaks after the two annual rainy seasons (April–June and October–December). Annual parasite index (API) was 6.5 per 1000 population in 2009 (Hormozgan Health Center, unpublished data).

2.2. Study design and distribution of bed nets

On the basis of available epidemiological data and average malaria incidence rate in the Hormozgan health center, fourteen villages with similar topographical and epidemiological situations were selected for implementation of bed nets. Villages were randomly assigned to 2 clusters of the study: Olyset net and untreated net.

Olyset area comprised eight villages with 1588 population and untreated nets area had six villages with 975 inhabitants. The study design is shown in Fig. 1.

The intervention phase started in September 2009 and continued up to August 2010.

The bed nets were distributed in September 2009, one month before the beginning of the second malaria transmission season. Nets were distributed and delivered free at health facilities to the all households in the selected villages according to the size of the family. In total, 720 Olyset nets and 464 plain nets were distributed in the study areas.

2.3. Specification of bed nets

The Olyset nets were manufactured by Sumitomo Chemical Co., Ltd. Nets were made of blue polyethylene monofilament fiber, 150 denier strength with 72 meshes (8 by 9 holes/in.²). The nets were blended with 1000 mg a.i/m² (2%, w/w). Untreated nets were made of white polyester polyfilament fiber, 100 denier strength with 156 meshes (12 by 13 holes/in.²). All nets (LLINs/untreated nets) were rectangular shaped and of extra family size (width, 180 cm; length, 190 cm; height, 150 cm). All the nets were supplied by the Ministry of Health and Medical education of the country.

2.4. Entomological evaluation

Entomological surveys were carried out monthly from September 2009 to August 2010.

Mosquito collections were made in and around two villages (fixed sites) in Bashagard district: Tisur (26°30′ N, 58°15′ E, 840 m) in Olyset net area and Daranar (26°15′ N, 58°23′ E, 420 m) in untreated net area, although random collections were made in twelve additional villages (variable sites) located in different
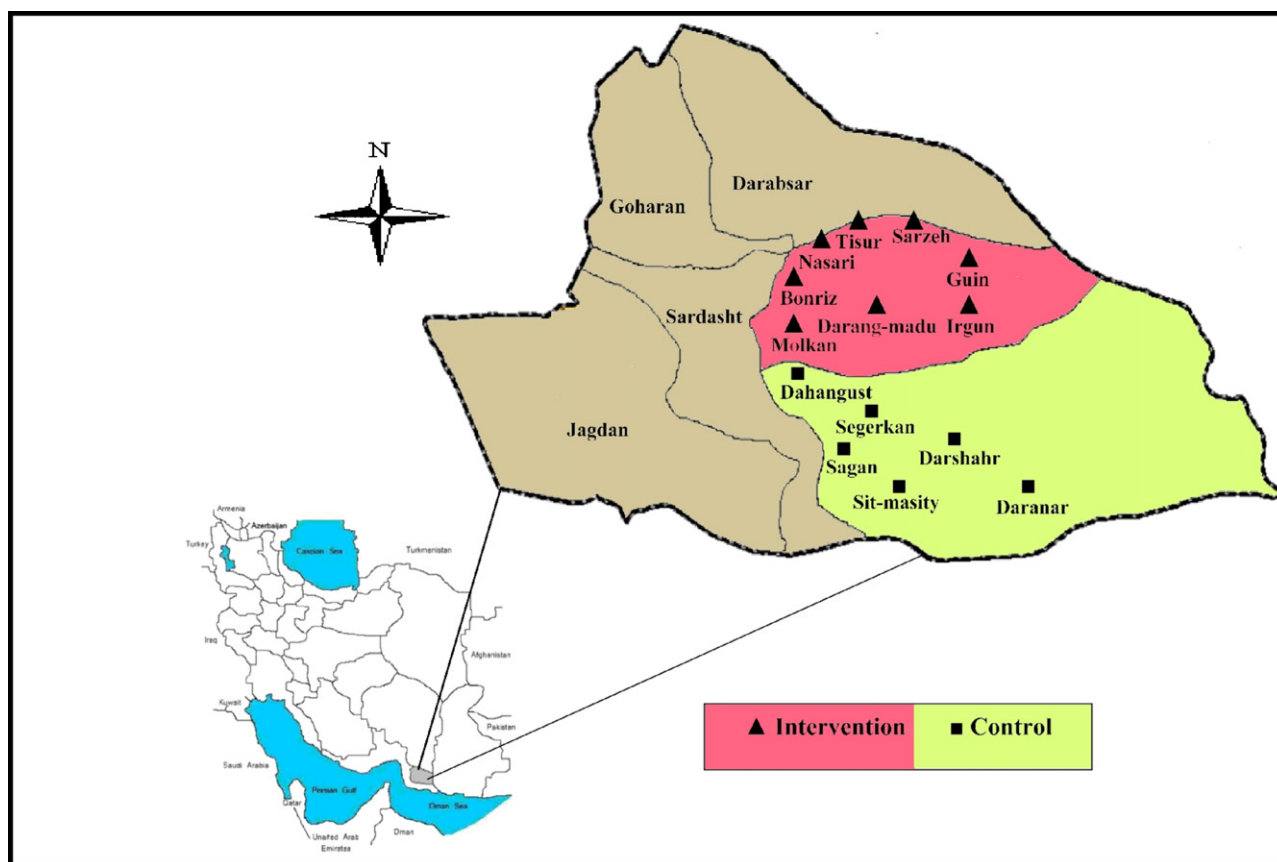
Fig. 1. Map showing the provinces of Iran, highlighting the position of Bashagard district villages (study villages).

topographical areas of the district (seven villages in Olyset net area and five villages in untreated area).

Indoor resting mosquitoes were collected by total catch (spray sheet collection) and hand catch (WHO, 1975). Adult mosquito density was measured in four houses per villages as fixed sampling and four houses selected randomly each in Olyset and untreated nets villages. Outdoor resting mosquitoes were collected by standard methods involving pit traps and night biting catch on donkey (Rozendaal, 1997). The specimens were identified using the morphological characters based standard key (Shahgudian, 1960).

2.5. Human blood index

To determine human blood index (HBI), blood fed mosquitoes were collected by hand catch from human dwelling and outdoor resting places (pit shelters). The blood meals of the identified anopheline were smeared on circle of Whatman filter paper No.1 (Whatman International Ltd., Kent, United Kingdom). Papers were sealed in plastic pages and kept in $-20\,°C$ until examined using enzyme-linked immunosorbent assay (ELISA), as described by Edrissian et al. (1971).

2.6. Residual analysis and bioassays

The persistence of insecticide on nets over the time was determined by chemical analysis and contact bioassay conical tests before bed nets distribution and thereafter at 3 months intervals during intervention period.

In each series of bioassay and chemical analysis, 10 Olyset nets and 10 plain nets were randomly sampled. The nets which were removed from each household for sampling were replaced with new nets. Two side-by-side pieces of netting ($25\,cm \times 25\,cm$) were removed from the middle of one of the longer sides of each net, placed in aluminium foil and stored in a cool, dark place until eventual chemical and bioassays analysis was performed as follows: The contact bioassay was performed according to the standard WHO test procedures by exposing laboratory rearing female *A. stephensi* (Beech strain), unfed, 3–5 days old and susceptible to all pyrethroids. Five mosquitoes were released in each bioassay cone simultaneously and exposed to netting samples for 3 min. Knockdown was measured in log time during 64 min post-exposure. Accordingly, mortality was measured after 24 h. At the end of the exposure time the mosquitoes were transferred to plastic cups, where they were provided with a piece of cotton wetted in 10% glucose solution during the recovery period. Mosquitoes were considered knocked down or dead if they could not fly and could not stand upright on either side or the bottom of the paper cups. If the mortality in controls was between 5 and 20%, the percentage mortality was corrected by the formula Abbott's formula (1925).

The tests were repeated ten times for each net sample, i.e. a total of 50 mosquitoes were tested per net. Plain nets were used as the LLINs. Bioassays were carried out at $25 \pm 2\,°C$ and $75 \pm 10\%$ relative humidity.

High performance thin layer chromatography (HPTLC) was used to measure permethrin residue on the Olyset nets. To assess the insecticide residue, $10\,cm^2$ pieces of Olyset nets were cut before use and during the intervention period. Net pieces were kept at $4\,°C$ for assuring the preservation and determining the residue of permethrin.

For extraction of permethrin residue from net pieces, 10 ml pure acetone was added to each vial containing $10\,cm^2$ pieces of netting. The insecticide residue was extracted by 10-min shaking and then allowed to stand 1 h just before analysis to ensure extraction of a representative quantity of permethrin. The extracts were

Table 1
Composition and localities of the adult anopheline mosquitoes collected from indoor and outdoor shelters in Olyset net and untreated net areas.

Study area	Species	Indoor			Outdoor			Total
		Hand catch	Total catch	*n* (%)	Pit shelter	Night biting catch	*n* (%)	*n* (%)
Olyset nets	*A. culicifacies*	20	112	132 (16.53)	37	69	106 (13.27)	238 (11.25)
	A. stephensi	8	54	62 (7.76)	54	78	132 (16.53)	194 (9.17)
	A. dthali	16	84	100 (12.50)	32	95	127 (15.90)	227 (10.73)
	A. superpictus	3	14	17 (2.13)	12	43	55 (6.88)	72 (3.41)
	A. fluviatilis	–	–	–	–	38	38 (4.75)	38 (1.80)
	A. moghulensis	–	–	–	–	12	12 (1.50)	12 (0.56)
	A. turkhudi	2	8	10 (1.25)	3	5	8 (1.00)	18 (0.85)
	Total	49	272	321 (40.17)	138	340	478 (59.83)	799 (37.77)
Untreated nets	*A. culicifacies*	12	212	224 (17.02)	25	118	143 (10.87)	367 (17.35)
	A. stephensi	32	104	136 (10.33)	38	140	178 (13.52)	314 (14.85)
	A. dthali	42	204	246 (18.70)	29	161	190 (14.44)	436 (20.62)
	A. superpictus	4	36	40 (3.04)	7	63	70 (5.32)	110 (5.20)
	A. fluviatilis	–	–	–	–	53	53 (4.03)	53 (2.51)
	A. moghulensis	–	–	–	–	16	16 (1.21)	16 (0.76)
	A. turkhudi	–	7	7 (0.53)	5	8	13 (0.99)	20 (0.94)
	Total	90	563	653 (49.62)	104	559	663 (50.38)	1316 (62.23)
Total	–	139	835	974	242	899	1141 (100)	2115

evaporated until the final solution volume was reduced to 1 ml. Two replicate extractions were performed for each treatment. The spotting on a silica gel containing aluminium plate was performed by an applicator and capillary tubes. Volume of each spot was 10 μl and the distance between spots was 1 cm. Ten mg of permethrin standard was mixed with 90 ml of pure acetone solution to produce 10% concentration. Then the solution was stored in glass-stopper bottles at 4 °C. Ten μl of extracted and standard solutions were spotted separately on a plate as a stationary phase. After spotting and drying the spots, the plate was put inside the TLC chamber tank. The mobile phase solvent was *n*-hexane–ethyl acetate (90 + 10, v/v). Plate was placed in the tank saturated with vapors of the developing solvent. Subsequently it was withdrawn from the tank and the mobile phase was evaporated after 30 min. After drying, spots were observed in UV cabinet by florescence light with 254 nm wavelength. Finally, the spots were scanned by TLC Scanner 3 (CAMAG), using CATS4 software (Gupta et al., 1998; Sherma, 2005).

2.7. Malaria surveillance

Malaria incidence was measured by passive and active case detection from all study population, one year prior to bed net distribution (September 2008 to August 2009), and again throughout the study period (September 2009 to August 2010). Thick and thin blood smears were made for all individual with a clinical finding suggestive of malaria. Presumptive treatment was given to all the fever cases, while radical treatment was given only to malaria positive cases. Data were pooled together for before and after intervention period.

2.8. Ethical considerations

The study protocol was approved by the ethical committee of School of Public Health, Tehran University of Medical Sciences.

2.9. Statistical analysis

The data were subjected into SPSS V. 16 and then were analyzed. The Student's *t*-test was used to determine the significance of difference in the different parameters between the Olyset nets and untreated nets. For calculating median knockdown time the Probit analysis of Finney (1971) was used. The results were considered significant at 5% level of significance ($P < 0.05$). Graphs were prepared using Excel software.

3. Results

3.1. Entomological evaluation

A total of 2115 adult anopheles mosquitoes were collected in the study area, including *A. culicifacies*, *A. dthali*, *A. stephensi*, *A. superpictus*, *A. fluviatilis*, *A. moghulensis* and *A. turkhudi*. *A. dthali* was the predominant vector species in both areas.

A. superpictus and *A. fluviatilis* were generally the least abundant among the seven species and found in two study villages. Other non-vector anopheline species were *A. moghulensis* and *A. turkhudi*. In this study *A. fluviatilis* and *A. moghulensis* were collected by night biting catch but *A. culicifacies*, *A. dthali*, *A. stephensi* and *A. superpictus* were collected by different methods. Indoor and outdoor population size for all months in untreated area was more than Olyset area (Table 1).

3.2. Density of malaria vectors

The impact of Olyset nets on the indoor-resting density of malaria vectors *A. culicifacies*, *A. stephensi*, *A. dthali* and *A. superpictus* is shown in Fig. 2.

There were 41.1%, 54.4%, 59.39% and 64.1% reduction in the density of *A. culicifacies*, *A. stephensi*, *A. dthali* and *A. superpictus* in houses with Olyset in comparison with houses with untreated nets, respectively during the study period. The total anopheline vector density was significantly lower in Olyset area compared with untreated area during the study period ($\chi^2 = 2.3$, df = 94, $P = 0.024$).

In both areas, malaria vectors showed a main peak of indoor-resting density during March–May and a smaller peak in September–December (Fig. 2).

3.3. Human blood index of malaria vectors

In total, 112 and 184 of collected mosquitoes were freshly fed and eligible for ELISA examination in Olyset net and untreated area, respectively. These comprised *A. culicifacies*, *A. dthali*, *A. stephensi* and *A. superpictus*. The overall 22 (19.64%) and 56 (30.43%) of the blood meals in Olyset net and untreated net areas were from human, respectively. In Olyset net area mosquitoes preferred outdoors more than human houses so that, the majority of blood-fed mosquitoes were collected from pit shelters (Table 2). Results also showed that, the HBI of malaria vectors substantially reduced in the Olyset net area in comparison to the untreated net area.
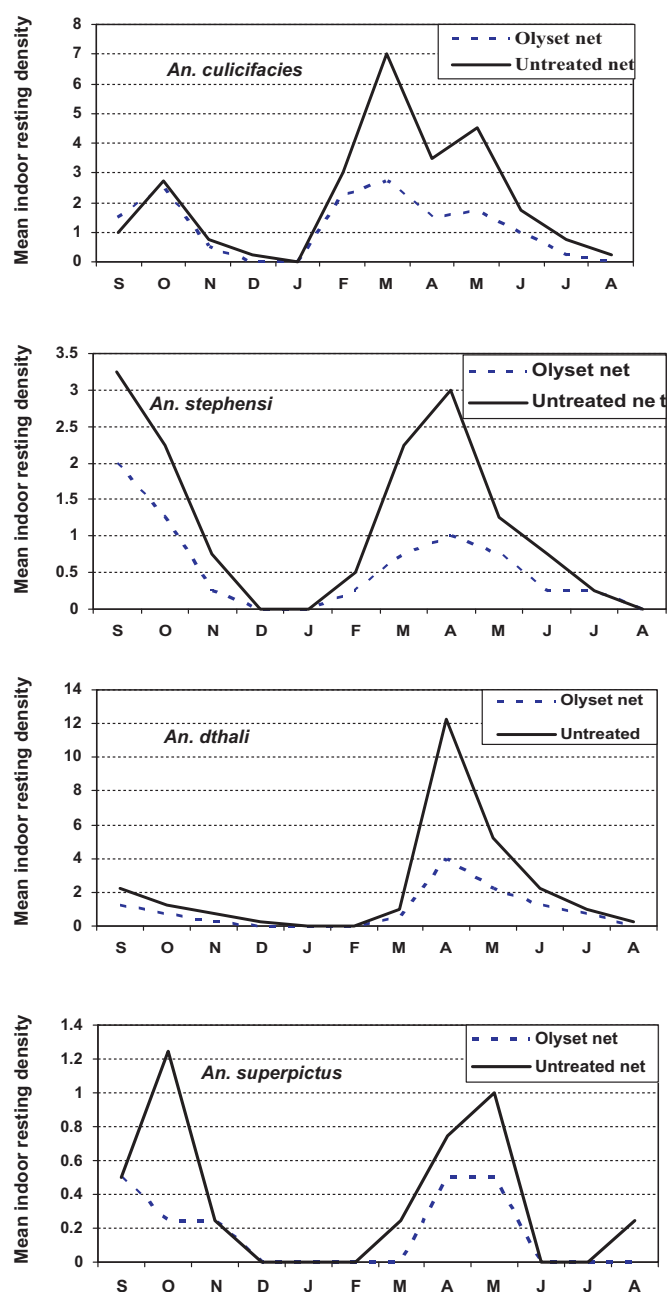
Fig. 2. The monthly mean indoor resting density of malaria vectors Olyset net and untreated net areas.

Generally, the human blood index for malaria vectors was significantly lower in trial compared with the untreated net area ($\chi^2 = 4.57$, df = 6, $P = 0.004$). The impact of Olyset nets on the human blood index of malaria vectors is shown in Fig. 3.

3.4. Residual analysis

The results of HPTLC analysis on Olyset nets before and during the intervention are shown in Table 3. The average permethrin content on Olyset nets was close to the target of 1000 mg a.i./m^2 in the first sample series. After 6 months of trial, 81.4.% of the initial mean dosage remained and at the end of trial the mean insecticide residue had decreased to 683.1 mg a.i./m^2. It should be mentioned that all of the net samples, which were collected randomly during the study period were unwashed.

3.5. Bioassay tests

Average 24-h post-exposure mortalities of *A. stephensi* exposed to Olyset nets before and after bed nets distribution are presented in Table 3. Olyset nets caused 100% mortality in *A. stephensi* with 3-min exposure, before and 3 months after nets distribution but the percentage of mortality gradually decreased to 72.3% after 12 months of use while no mortality was recorded in *A. stephensi* on untreated nets.

The results of median knockdown time are shown in Table 3. There was a gradual increase in the median knockdown time from 1.48 to 3.52 min, before distribution and 12 months of use, respectively. The increase in median knockdown time was significant ($P < 0.05$).

Results also showed that, the median knockdown time was strongly correlated with the amount of permethrin detected on the nets using HPTLC (Fig. 4).

3.6. Malaria incidence

Data on malariometric indices such as blood slid examined (BSE), slid positive rate (SPR) and annual parasite index (API) in two clusters of villages during the pre-intervention and intervention period are shown in Table 4.

The parasite index during the pre-intervention period in the Olyset net area and untreated area was 74.7 and 104.9 cases per 1000 population, respectively. The incidence of malaria was similar in two study areas and no statistically significant difference was observed ($P > 0.05$).

During the intervention period, the malaria incidence in the Olyset villages had come down drastically. The annual parasite index in the Olyset net and untreated net population was 2.52 and 36.9 respectively. There was a reduction of 93.2% in malaria incidence in the Olyset net area as compared to the untreated net area.

Table 2
Human blood index (HBI) of anopheline vectors according to resting places in Olyset net and untreated net areas.

| Study area	Species	Indoor		Outdoor		Total	
		Tested	HBI (%)	Tested	HBI (%)	Tested	HBI (%)
Olyset nets	*A. culicifacies*	18	3(16.6)	28	6(21.4)	46	9(19.56)
	A. stephensi	5	1(20)	29	5(17.2)	34	6(17.64)
	A. dthali	10	3(30)	10	2(20)	20	5(25)
	A. superpictus	3	1(33.3)	9	1(11.1)	12	2(16.66)
	Total	36	8(22.22)	76	14(18.42)	112	22(19.64)
Untreated nets	*A. culicifacies*	22	8(36.4)	13	5(38.5)	35	13(37.14)
	A. stephensi	39	12(30.8)	7	1(14.3)	46	13(28.26)
	A. dthali	73	24(32.9)	12	2(16.6)	85	26(30.58)
	A. superpictus	16	4(25)	2	0(0)	18	4(37.5)
	Total	150	48(32.00)	34	8(23.52)	184	56(30.43)
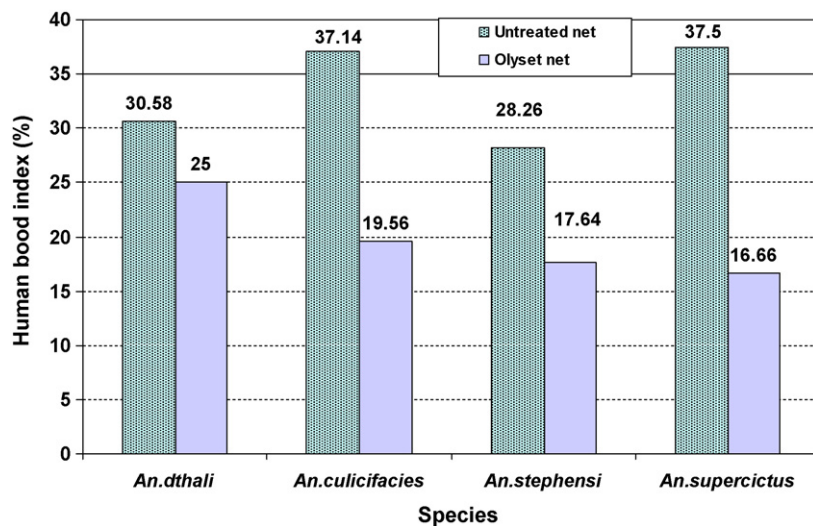
Fig. 3. Human blood index of vector species in Olyset net and untreated net areas.

Table 3
Permethrin residue (mg/m^2) assessed by HPTLC and bioafficacy of Olyset against *A. stephensi* after 3 min of exposure and 24-h recovery period.

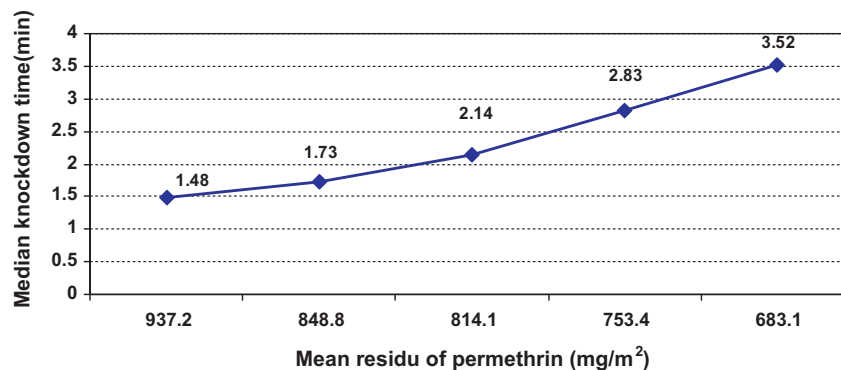| Status	Month	No.	Mean concentration (mg/m^2) (95% C.L.)	SD	SE	Median knockdown time (min)	Mortality (%) ± error bar
Pre-intervention	0	10	937.2	21.69	6.86	1.48	100
Intervention	3	10	848.8	32.76	10.36	1.73	100
	6	10	814.1	34.39	10.87	2.14	92.4 ± 4.23
	9	10	753.4	40.28	12.73	2.83	81.6 ± 6.18
	12	10	683.1	37.91	11.98	3.25	72.3 ± 7.07

Fig. 4. The relationship between median knockdown time (min) and mean residue of permethrin in 50 Olyset nets collected in field and assessed by HPTLC.

Compared with the baseline data, the reduction in malaria incidence in the population using Olyset nets and untreated nets was significant. However, the difference in malaria incidence between two study areas during the intervention period was highly significant (*P* < 0.01).

4. Discussion

This is the first formal report for field evaluation of Olyset nets as a part of large scale LLINs programme in Bashagard district, south of Iran.

Table 4
Malariometric indices in the villages with Olyset nets and untreated nets during pre-intervention and intervention period.

Year	Study area	Population	BSE	Total malaria cases	*P.v*	*P.f*	SPR (%)	API
Pre-intervention (Sep. 2008–Aug. 2009)	Olyset nets	1538	1363	115	115	0	8.44	74.7
	Untreated nets	943	777	99	99	0	12.74	104.9
Intervention (Sep. 2009–Aug. 2010)	Olyset nets	1588	1474	4	3	1	0.27	2.5
	Untreated nets	975	836	36	36	0	4.30	36.9

BSE, blood slid examined; *P.v*, *Plasmodium vivax*; *P.f*, *P. falciparum*; SPR. slid positive rate; API, annual parasite index (no. malaria cases/1000 population).

During the study seven species of *Anopheles* were identified in study area. The most abundant adult anopheline mosquitoes were *A. dthali*, *A. culicifacies* and *A. stephensi* respectively. Previous studies have shown that *A. stephensi* is primary vector and other anopheline species, such as *A. culicifacies*, *A. dthali*, *A. superpictus* and *A. fluviatilis*, play the main role as secondary vectors in malaria transmission in Hormozgan province (Vatandoost et al., 2006, 2004; Emami et al., 2007; Oshaghi et al., 2007; Naddaf et al., 2003).

Results of this study revealed a significant reduction in indoor and outdoor total density of anopheline vectors in Olyset net area compared with untreated net area. Similarly, in studies which were conducted in India, density of mosquitoes in houses with Olyset nets was drastically reduced when compared to houses with untreated nets (Ansari et al., 2006; Sharma et al., 2009a). Many studies in the other malarious countries have recognized the high efficacy of Olyset in reducing indoor-resting density of anopheline mosquitoes (Ansari et al., 2006; N'Guessan et al., 2001; Sharma et al., 2009a; Sreehari et al., 2007). This reduction could be explained by the reduction in longevity of mosquito population due to mass killing, as demonstrated clearly in India, Gambia and Tanzania (Magesa et al., 1991; Quinones et al., 1998; Sharma et al., 2009a). Another possible explanation for the decrease of indoor-resting density of malaria vectors in trial area is exito-repellent effect of Olyset on the vector population.

Another important finding of this study was a significant decrease in the HBI of malaria vectors in Olyset net villages in comparison to untreated net villages. In a similar study conducted by N'Guessan et al. (2001) in Cote d'Ivoire, Olyset performed better than nets conventionally treated with permethrin in reducing man-vector contact as they significantly reduced entry and human blood feeding rates. The decrease of HBI, following the use of permethrin-treated bed nets has been reported in Tanzania, India and Burkina Faso (Chandre et al., 2010; Malima et al., 2008; Maxwell et al., 2006). This may be due to exito-repellency effect of permethrin that increase the probability of mosquitoes to exit homes and cause animals to be an alternative blood meal source.

In this study, the median knockdown time increased from 1.48 min at baseline to 3.25 min on Olyset after 12 months of use in the field conditions. Recent studies carried out in India and Tanzania support our findings (Sharma et al., 2009a; Tami et al., 2004). The knockdown time is directly correlated to the insecticide concentration over the surface for fast-acting insecticides such as pyrethroids (WHO, 1998). This also implies that regeneration of insecticide over the surface of nets is very fast. This may be because of hot tropical conditions which cause migration and replenishment of permethrin to the surface of net fibers more quickly. Bioassay gives an estimate for the insecticide on the surface of the net fibre that can potentially be absorbed by mosquitoes and determines the time period required for the regeneration of a LLINs after repeated washing (WHO, 2005). Bioefficacy increment in WHO cone bioassays is probably associated with increased protection from anopheline biting. Therefore, it is important to aim for maximum efficacy, in terms of bioassay mortality and knockdown, to achieve maximum protection against malaria under field conditions (Gimnig et al., 2005).

In the present study, the average permethrin concentrations after one year of use was effectively high (683.1 mg/m^2) and Olyset nets were found effective and provided 72.3% mortality against *A. stephensi* in bioassay tests. The recommended dosage for permethrin on conventional nets is 200–500 mg/m^2. Thus, the permethrin concentration on these nets is theoretically adequate to be effective.

Long-lasting nets must achieve equilibrium where much of the insecticide is retained within the fibre or resin while enough migrates to the surface of the net where it is available to kill or knockdown mosquitoes (Gimnig et al., 2005). Our results also are similar to findings of N'Guessan et al. in Cote d'Ivoire (2006). Who

reported good insecticide persistence after 3 years of use and Olyset had high bioefficacy inducing >80% mortality in bioassay when tested in experimental huts. In trials in India, it was reported that 3-min exposure to Olyset induced 100% mortality in wild-caught blood-feed *A. culicifacies* and *A. fluviatilis* after 11 and 20 washings, respectively (Sharma et al., 2009a). In another study carried out in Tanzania, after seven years of continuous use, the permethrin content of Olyset was still 35% of the initial loading dose and 90% of the nets were still fully active against *A. gambiae* in terms of knockdown in 3-min exposure bioassay test (Tami et al., 2004).

Present study has shown a considerable reduction of malaria incidence following distribution of bed nets with more reduction in Olyset net area compared to untreated net area. This reduction is probably a result of repellent and killing action of the Olyset on malaria vectors. Similar findings have been reported from rural area of the India (Sharma et al., 2009b; Sreehari et al., 2007). Many studies in the other endemic countries have demonstrated reduction in malaria incidence following the introduction of permethrin treated bed nets (Snow et al., 1988; Beach et al., 1993; Stich et al., 1994).

Our results indicated that Olyset provide high efficacy against malaria vectors, including reduction in indoor-resting density of anopheline mosquitoes and increment of personal protection, as measured by reduction of human blood index. Moreover, these long-lasting nets retained high levels of insecticide and good performance as well as quick knockdown and high mortality in WHO cone bioassays. In the present study Olyset were monitored for only 12 months after distribution. Future studies are required to investigate the long-lasting efficacy of these LLINs over at least three years in the field conditions.

Conflict of interest

The authors declare that have no conflict of interest.

Author's contributions

MSA has designed the study, coordinated field activity, collected data, trained field researcher and drafted the manuscript. HV designed and supervised the study and revised the manuscript. MS, AR and FA participated in the conception of study design. MRE performed the statistical analyses. The field research activities were supported by RS. MAO supervised the laboratory test. MA carried out the HPTLC chemical analysis. HH carried out the ELISA assays. All authors read and approved the final manuscript.

Acknowledgements

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