

Efficacy Trial of New Pest Control Product: Olyset Plus-Long Lasting Insecticide Treated Net with Added Synergist (Piperonyl Butoxide) for Malaria Vector Control in Kenya

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Abstract

Background: Scientists and Manufactures are working closely in order to develop new tools and strategies that can fight resistant malaria vectors more efficiently. Sumitomo chemicals of Japan a company with a long history of production of long-lasting insecticides treated nets (LLINs); Olyset® nets have developed Olyset® Plus, which has synergy- piperonyl butoxide (PBO). It is anticipated while PBO is not an insecticide; it will enhance the knockdown effect of permethrin on olyset net thus killing mosquitoes with metabolic resistance mechanism. The study aimed at evaluating efficacy trial of new pest control product: Olyset® Plus-long lasting insecticide treated net with added synergist (piperonyl butoxide) for malaria vector control in Kenya.

Methodology: We evaluated the knockdown and killing effect of Olyset® Plus net compared to Olyset® net which is within the Kenyan market using field collected mosquitoes.

Results: The Olyset® Plus nets showed 100% mosquito knockdown indicating good performance compared to Olyset® a locally marketed LLINs which had 95%. Permethrin-treated net with PBO was found to be more effective against mosquitoes, retains acceptable mosquito killing effect after several washes.

Conclusion: Olyset® Plus nets are recommended for registration and use in Kenya as an LLINs against mosquito vectors of malaria if it meets all other requirements of Pest Control Products Board.

Keywords: Olyset plus; Malaria; Mosquitoes; Pest control

Introduction

The scale-up of malaria interventions particularly malaria vector control has gained popularity within the last twenty years with the initiation of roll back malaria initiative in 1998 and the development of insecticide-treated mosquito nets in the 1980s. The current reduction of malaria burden depends greatly on the use of artemisinin combination therapy and the massive use of insecticide-treated mosquito nets (ITNs), supported by preventive treatment strategies during pregnant and indoor residual spraying [1]. Nonetheless, malaria is still a major public health problem in sub-Saharan Africa, affecting 200 million people annually resulting in half a million deaths annually [2]. Resistance mechanisms of Plasmodium parasite against antimalarials and Anopheles vectors to insecticides have greatly challenged malaria control tools [3,4].

Mosquito nets have evolved over a period ranging from untreated nets to manually treated nets and finally, factory treated nets referred to as long-lasting insecticidal nets (LLINs). This development has evolved due to discoveries of pyrethroids, insecticide class with low mammalian toxicity [5]. The national malaria control programmes (NMCP) in the African region have embraced LLINs as the main tools for malaria vector control [5-7]. In Kenya, NMCP has distributed LLINs with a significant reduction in malaria morbidity and mortality in all malaria epidemiological zones. For example, net distribution has reduced malaria prevalence around Lake Victoria basin from 38% in 2010 to 28% in 2016 (KMIS, 2015). The gain achieved in net distribution may be compromised by widespread of pyrethroids resistance. The fear of loss in gain in malaria prevention due to mosquito resistance to pyrethroids is highlighted by WHO in the global programme for insecticide resistance management strategic plan (GPIM), which has advised member countries to sub guard and use techniques to delay

and manage insecticide resistance [5-7]. Kenya is in the process of adopting these recommendations and developing her insecticide resistance management strategic plan. Meanwhile, private sectors led by Innovative Vector Control Consortium (IVCC) and World Health Organization Pesticide Evaluation Scheme (WHOPES) are in the forefront in exploring new insecticide formulations, insecticide mixtures and other techniques which could be adopted by malaria-endemic countries to overcome the problems of insecticide resistance [8].

Sumitomo chemicals of Japan a company with a long history of production of LLINs; Olyset® net has developed Olyset® Plus, which has a synergist-piperonyl butoxide (PBO). It is anticipated while PBO is not an insecticide; it will enhance the knockdown effect of permethrin on Olyset® net thus killing mosquitoes with metabolic resistance mechanism [9-11]. As a part of registration requirement of this new product, we have evaluated the knockdown and killing effect of Olyset® Plus net compared to Olyset® net which is within the Kenyan market using field-collected mosquitoes. This is with reference to the Pest Control Products Board request: Permit No. PCPB/112Eval/VOL.1/15/223 dated 18th May 2015.

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Received December 19, 2018; Accepted February 09, 2019; Published February 15, 2019

Citation: Wangai LN, Njagi K (2019) Efficacy Trial of New Pest Control Product: Olyset Plus-Long Lasting Insecticide Treated Net with Added Synergist (Piperonyl Butoxide) for Malaria Vector Control in Kenya. J Med Microb Diagn 8: 290. doi:10.4172/2161-0703.1000290

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Materials and Methods

Study area

The trial was conducted in the VBDCU (Vector-Borne Disease Control Unit) laboratory located within Kimbimbi sub-county hospital in Mwea, Kirinyaga County. This health facility serves the community of Mwea irrigation scheme an area located at 1150 m above the sea level. The facility also houses a regional entomology laboratory with enough trained personnel and equipment for conducting various tests including testing for efficacy of insecticides and insecticide-treated materials used in public health for vector control.

Trial materials

For the purpose of this trial, olyset plus nets were provided by Sumitomo chemicals of Japan through Quest chemicals. The LLINs were stored in the laboratory at room temperature to mimic the actual user's condition. LLINs were then washed regularly and allowed to dry at room temperature up to 21 washes. The washed nets were marked using a felt pen indicating the number of washes per each batch of nets.

Field mosquitoes used for the trial were collected from houses within the irrigation scheme using mouth aspirators. The collected mosquitoes were kept in the laboratory for 24 hours to acclimatize after which healthy individual mosquitoes selected and used for the tests.

Details of the product:

Trade name: Olyset plus.

Formulation: Long lasting insecticide treated nets (LLINs).

Active ingredient: Combination of Permethrin and piperonyl butoxide.

Concentration of active ingredient: Permethrin 20 g/kg plus Piperonyl butoxide 10 g/kg.

Chemical class: Pyrethroids plus non-insecticide piperonyl butoxide.

Use: Indoor sleeping spaces.

Target vectors: Indoor adult biting mosquitoes.

Manufacturers: Sumitomo chemicals of Japan.

Local agent: Quest Chemicals, PO BOX 18686-00100, Nairobi, Kenya.

Recommended intervals of application: Three years of use or 21 washes.

WHO cone bio-assay

WHO standard cone bio-assay was conducted on batches of Olyset® Plus nets based on the number of washes, ranging from 0 wash (unwashed net) to a batch of nets washed for 21 times. Briefly, cones were attached at different sites within a single net using rubber buds. The choice of the sites was carefully selected to represent the top of the net, lower edges and the middle of the net as washing and dripping may have effects on distribution of chemicals on netting materials. Similarly, the positive and negative controls were set using untreated nets and Olyset® net commonly found within the local supermarket.

Using mouth aspirators approximately 18-20 mosquitoes were picked from the holding cages and introduced into the cone. The cones were then plugged with a ball of cotton wool.

The mosquitoes were allowed to remain in the cone (in contact with the trial net) for three minutes after which they were removed using mouth aspirators and placed in paper cups.

The exposed mosquitoes were observed for 60 minutes to determine the knockdown rate of Olyset® Plus net as compared to Olyset® net and the untreated net. The observations were carried out at time intervals of 5 minutes, 10 minutes, 15 minutes, 30 minutes, 45 minutes and 60 minutes. Data on knockdown, dead and live mosquitoes were recorded in a structured reporting form. At the end of the 60th minute, all exposed mosquitoes regardless of their knockdown status were left in the paper cups, fed on 10% sugar solution and a further observation was done at 24 hour. The 24 hours mosquito mortality was recorded. Both one-hour mosquito knockdown and 24 hours mosquito mortality were used to determine the efficacy of Olyset® plus net as compared to Olyset® net and untreated net. For the purpose of data analysis, the collected data was entered in MS Excel sheet and analyzed for consistency comparing the two LLINs on mosquito mortality and knockdown.

Statistical analysis

Results from the WHO cone bioassays were compared between each net using a Chi square test. Data were analyzed using the STATA version 16. P values of less than 0.05 were considered significant. The mosquitoes count entering the huts was compared and analyzed using the non-parametric Kruskal-Wallis test and the parametric t-test.

Results

The ability of the net to protect the users by killing mosquitoes landing on it was estimated by observing mosquito knockdown within the first 60 minutes and 24 hours mortality as per WHO criteria. The ability to retain the insecticide after several washes were determined similarly by observing mosquito knockdown after 60 minutes and 24 hours mortality of nets washed in ranges of wash 1 to wash 21 as per WHO definition of long-lasting insecticide-treated nets.

Bio-assay results for Olyset® Plus at 0 wash (before the start of washing) and the 21 wash are as shown in Figure 1.

The nets showed 100% mosquito knockdown indicating good performance compared to Olyset® net a locally marketed LLINs which had 95%.

With 21 washes, Olyset® Plus net and Olyset® net showed a knockdown rate of 97% and 90% respectively indicating enough insecticide to immobilize mosquitoes was retained on the surface of both nets.

Wash resistance, the ability of Olyset® Plus net to retain permethrin with or without PBO after several washes were confirmed as nets washed for over 20 washes still retained ability to immobilize the mosquitoes landing/exposed on them. However, Olyset® Plus had a faster knockdown rate and higher knockdown percentage compared to Olyset® net indicating the importance of PBO to enhance knockdown and killing effect of permethrin on Olyset® Plus nets.

Observation of 24 hours mosquito mortality

One of the key parameters on the determination of efficacy of LLIN is the ability of the net to continuously kill mosquitoes 24 hours post exposure. After 24 hours dead mosquitoes are unlikely to recover compared to 60 minutes knockdown where some mosquitoes might regain and fly off.

The 24 hours mosquito mortality was observed in both Olyset® Plus

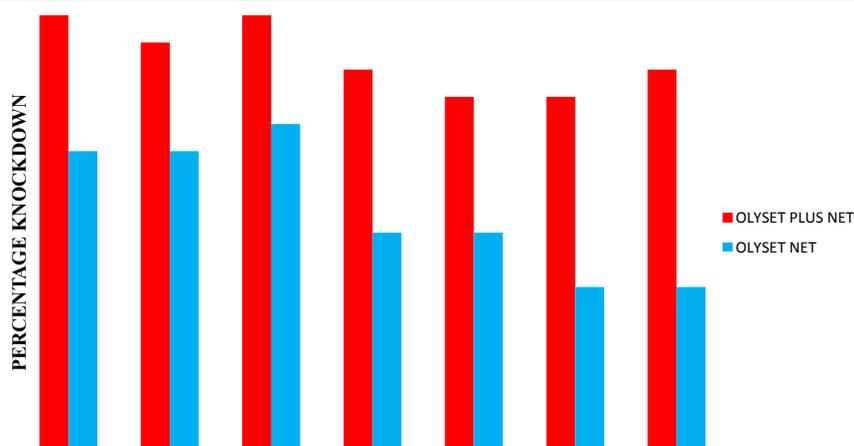


Figure 1: Comparison of mosquitoes knockdown (60 minutes post exposure) of the two LLINS- Olyset plus net and Olyset net per wash round.

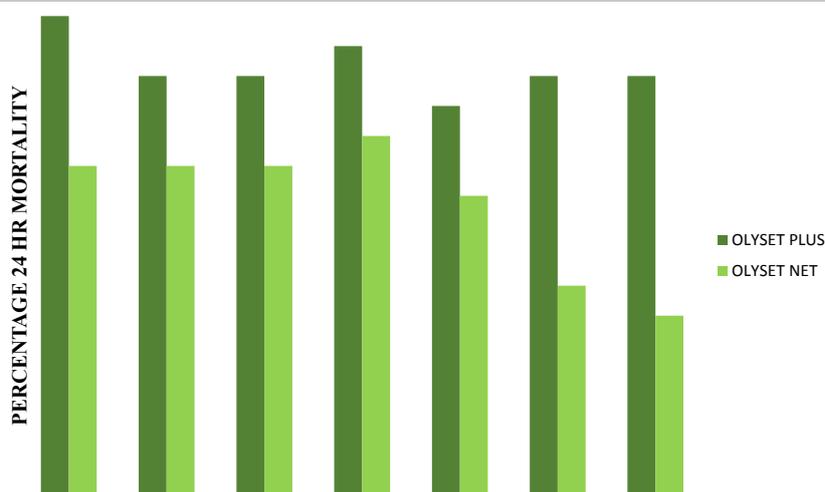


Figure 2: Comparison of mosquito mortality (24 hours post exposure) of two LLINS- Olyset plus net and Olyset net.

Net and Olyset® Net an LLIN commonly marketed in Kenya in the last 12 years (2004 to date). Data collected on 24 hours mosquito mortality indicated both nets achieved relatively higher mortality of 100% for Olyset® Plus net at 0 wash compared to 95% of Olyset® Net (Figure 2). These figures were higher compared to WHO cut off 80% mortality). Comparing the two nets at 21 washes, 98% for Olyset® Plus net and 90% Olyset® Net were again above acceptable WHO cut off 80%. Variation within washes showed that Olyset® Plus Net was superior to Olyset® Net though not statistically different ($p=0.1934$).

Discussion

The prevailing theory concerning the combination of PBO and pyrethroid relies on the metabolic enzyme inhibitory effect of PBO. This combination might enhance the efficacy of pyrethroids against mosquitoes in areas with metabolic resistance. Furthermore, studies have shown that through its action on enhancing cuticular penetration of deltamethrin it can act as an adjuvant [9]. The strategy for enhancing the synergistic effect has demonstrated promising advances against pyrethroid-resistant malaria vectors. The findings demonstrated that permethrin treated net with PBO has more effective against mosquitoes and retains acceptable mosquito killing effect after several washes as compared to the standard Olyset® Net which is within the Kenyan market.

In the parent study, permethrin treated net with PBO performed better than the standard Olyset Net® in terms of killing effect against the mosquito population. The results confirmed the good efficacy of Olyset® Plus regardless of the presence or absence of resistance. This result is consistent with the recent past studies conducted in Benin and Cameroun where Olyset® Plus resulted to a higher mortality rate than Olyset® Net against the resistant populations, thus indicating the significant synergistic effect of PBO [10-12]. The additional effect of PBO has also been demonstrated on a roof panel where LLIN containing deltamethrin and PBO was compared with deltamethrin solely. However, in contrast to this study, the former study used a different technique, deltamethrin contents and Fabrics in manufacturing the LLIN, resulting to difficulties in interpretation of the findings [10,13]. In this study, Permethrin treated net without PBO was used as the positive control and indeed the efficacy, benefits and availability of Permethrin treated net with PBO was demonstrated clearly.

The study also emphasized on the ability of Olyset® Plus treated net to retain the insecticide after several washes by observing mosquito knockdown after 60 minutes to 24 hours and after 21 washes as per WHO definition of long-lasting insecticide-treated nets. Higher efficacy and protection were shown by Olyset® Plus Net as compared to the standard Olyset Net® in terms of insecticidal activity and long-

lasting protection. The present study agrees with the previous results which showed that Olyset® Plus Net possess higher efficacy than the standard Olyset Net® however, a lower exophilic activity was reported in Olyset® Plus nets as compared to the standard Olyset® Net. The study also confirmed the durability of the protective efficacy of the combination product to be higher than the Olyset® Net. The additional benefit of combining PBO with a pyrethroid insecticide into net has been evidently shown in this study, however, it is worth noting that it is very essential to develop more different tools other than LLINs and indoor residual spraying in targeting malaria vectors as more different evidences of mosquito behavioral changes have been reported [10,12-14]. These mosquito behavioral changes might render all these combination products useless.

Conclusion

Based on data generated on Olyset® Plus, a permethrin treated net with PBO was found to be more effective against mosquitoes, retains acceptable mosquito killing effect after several washings. Olyset® Plus net may therefore be considered as an LLIN, suitable for malaria control in Kenya. Addition of PBO to permethrin maybe an added advantage in this era of pyrethroid resistance. High levels of resistance have been reported particularly in Lake Victoria region where combination nets such as Olyset® Plus may be deployed.

Recommendation

We recommend Olyset® Plus nets, for registration and use in Kenya as an LLIN against mosquito vectors of malaria if it meets all other requirements of Pest Control Products Board.

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