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Effect of Netting Fabrics Impregnated with Different Doses of Natural Pyrethrins-Formulation against *Anopheles gambiae*

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ABSTRACT

Effect of different netting fabrics impregnated with varying doses of a stabilized natural pyrethins-emulsifiable concentrate formulation on bio-efficacy against *Anopheles gambiae* s.l. was investigated. Three-day-old female *An. gambiae* s.l. mosquitoes were exposed for three minutes on netting fabrics of nylon, polyester and cotton impregnated with the pyrethrins-formulation at 100mg/m^2, 250mg/m^2, 500mg/m^2 or 1000mg/m^2. Results demonstrated that, there was an overall mosquito knockdown of 45.6% and mortality of 14.0% on cotton as compared to 78.3% knockdown and 61.3% mortality on polyester and 90.4% on both knockdown and mortality on nylon fabric at 60 minutes and 24 hours post-treatment, respectively. There was a corresponding increase in knockdown and mortality with increasing dosage, though the magnitude depended on the impregnated fabric. The dosage of the pyrethrins–formulation required to achieve 80% mortality on mosquitoes when applied on a nylon net was 200mg/m^2 relative to polyester fabric that needed 375mg/m^2. It was, however, not possible to achieve the 80% mortality level with the impregnated cotton fabric at the test doses. These results demonstrate the significance of the intrinsic fabric properties and dosage level of the insecticide used for impregnation on the bio-efficay of natural pyrethrins in insecticide-treated nets (ITNs). Findings further show that the pyrethrins formulation was most suitable for treatment of nylon and polyester fabrics with a rather poor performance on cotton nets. These results are considered encouraging especially for efficient use of natural pyrethrins-formulation in ITNs.

KEY WORDS: Netting fabrics, natural pyrethrins-formulation, insecticide treated nets *Anopheles gambiae* s.I.

INTRODUCTION

Insecticide-treated net (ITN) trials undertaken in many African countries have shown remarkable success in reduction of overall malaria mortality and morbidity attributable to the use of this technology (Alonso *et al.,* 1991; Lines, 1996). Several studies have demonstrated that treatment of bed-nets with insecticide makes the nets more effective in preventing or deterring mosquito bites (Langeler, 2004; Hill *et al.,* 2006; Killeen and Smith, 2007; Killeen *et al.,* 2007). The ITN method employs low technology and is, thus, applicable at household level with minimal additional inputs.

Currently, there are various netting fabrics and insecticide formulations that are used in ITNs. The most common fabrics are polyester, nylon and cotton (WHO, 2006; Murari *et al.,* 2007). For ITNs, the WHO pesticides evaluation scheme recommends only pyrethroid insecticides in various formulations such as emulsifiable concentrates (EC), suspension concentrates (SCs), emulsion oil in water (EW) and micro-capsules (Zaim *et al.,* 2000). While studies elsewhere, have shown that differences in bio-efficacy of ITNs may closely be linked to the nature of fabrics, among other properties (Rozendaal, 1989; WHO, 2006; Rafinejad *et al.,* 2008), differences in results of various studies point out to the possible link between specific insecticide formulation and the empirical bio-efficacy (Hossain *et al.,* 1989; Lines, 1996; Vatandoost *et al.,* 2006). In another study, the efficacies of permethrin-treated nylon and cotton nets were compared and it was observed that 2.5 g/m^2 on nylon and 5.0 g/m^2 on cotton was required to prevent mosquitoes from

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biting humans (Hossain *et al.,* 1989). It was further demonstrated that cotton nets was not as effective as nylon nets when treated with permethrin against *Anopheles gambiae* and *An. funestus* (Lines *et al.,* 1987). However, in a separate study, it was demonstrated that polyester net gave maximum mortality over nylon and cotton nets when treated with lambda-cyhalothrin against *An. stephensi* (Vatandoost *et al.,* 2006). In general, cotton absorbs more emulsion than nylon and polyester nets, which are light and flexible and, therefore, take less insecticide emulsion (Rafinejad *et al.,* 2008). The net texture has also been observed to influence the choice of nets by users (Murari *et al.,* 2007) although with little consideration to its influence on bio-efficacy of the nets. Therefore, to improve on the use of ITNs in malaria control, a better understanding of the interaction between intrinsic fabric properties, formulation,impregnation dosage and empirical bio-efficacy of nets is necessary (MacCormack and Snow, 1986; Rafinejad *et al.,* 2008).

Furthermore, there is minimal information on the influence of the type of netting fabric and varying impregnation dosage on bio-efficacy of natural pyrethrins formulation-treated bed-nets. Pyrethrins have been said to be photo-labile (Casida and Quistard, 1995) and would therefore not be used successfuly on impregnated fabrics. However, when put in a suitable formulation with an anti-oxidant and a synergist the bio-persitence of pyrethrins is increased. Indeed, stabilized pyrethrins have been observed to have more than 80% mortality on mosquitoes for over six months when impregnated on a cloth (Warui and Mutinga, 1994). In addition, evaluation of the effectiveness of this formulation against mosquito vectors would be crucial in its usage in malaria control, especially with reported cases of resistance to synthetic pyrethroids (Hargreaves *et al.,* 2000; Ranson *et al.,* 2000). Therefore, this study investigated the influence of the type of netting fabric impregnated with different doses naturally-stabilized pyrethrins-formulation on bio-efficacy against *An. gambiae* s.l. mosquitoes.

MATERIALS AND METHODS

Test mosquitoes.

The laboratory tests were carried out on 3-day-old female *An. gambiae***,** Kisumu strain, reared at Kenya Medical Research Institute (KEMRI) - Centre for Global Health Research (CGHR) laboratory. The rearing employed previously described procedures for breeding the mosquitoes (Kamau *et al.,* 2006; Kamau and Vulule, 2006).

Formulation of a stabilized pyrethrins-emulsifiable concentrate.

An emulsifiable concentrate (EC) containing 5% pyrethins weight by volume (w/v) as the active ingredient (a.i) mixed with a synergist (25%), food grade anti-oxidant (3%) and a non-ionic emulsifier was formulated at the Pyrethrum Board of Kenya (PBK) laboratory based on established procedures. The active ingredient was obtained from a 25% pyrethrins (w/v) refined extract manufactured at the PBK factory while the synergist, emulsifier, and anti-oxidant were commercial grade obtained from manufacturers [Endura Spa (Italy) and Bayer AG (Germany)]. The active ingredient content in the formulation was determined to be $5\pm$ 0.5% through high performance liquid chromatography (HPLC) using a Varian Vista series 5000 liquid chromatography. In the analysis, 0.1g of the sample was put in 100ml volumetric flask and hexane added to the mark. The mixture was thoroughly shaken to achieve homogeneity then 10µL was injected into the system manually at the set flow rates (8ml/min, peak detector at 230nm, column factory packed CN10, run-time 10min and chart speed at 0.25cm/min). The % pyrethrins were then determined based on the shape and retention time of the peaks on the chromatogram and quantified against the curves of analytical world standard pyrethrum extract (WSPE).

Netting materials and impregnation.

Three multi-filament rolls of netting fibres of nylon, polyester and cotton of 100 deniers strength with mesh size of 25 holes/cm^2 (Siam Dutch Mosquito Netting Company, Thailand), were purchased from local stores in Kisumu City and used in this study. Pieces of nets measuring 2m^2 were cut and, before impregnation, their liquid absorbency was first determined to be 70ml/m^2 for nylon, 75ml/m^2 for polyester and 225 ml/m^2 for cotton by dipping a sample in water. The volume absorbed was measured by weight after normal wringing. Before impregnation, the amount of stock solution (X) of pyrethrins 5% emulsifiable concentrate (EC) needed to deposit a target dosage was determined as follows:

$$X = \frac{\text{Target dosage in (mg/m}^2\text{) x Area of the net (m}^2\text{)}}{\text{Conc. of stock insecticide g/kg}}$$

In order to impregnate the nets to deposit the respective doses equivalent to 100mg/m^2, 250mg/m^2, 500mg/m^2 and 1000mg/m^2, the following amounts of stock pyrethrins EC were used, i.e. 4 ml, 10 ml, 20 ml and 40 ml, respectively. The positive control was permethrin 20% emulsifiable concentrate (PERIPEL^{TM} 20 EC, Agrevo, formerly Russel Uclaf) at the recommended dose of 500 mg/m^2 .

The nets were then carefully soaked in a non-absorbent plastic bucket containing the mixed concentrate in a 'dip-it-yourself' manner. The fingers were protected with plastic gloves and impregnation properly executed in order to ensure uniform distribution of the solution onto the net. The nets were then left to dry for one-day on a glass surface in a room away from direct sunlight under ambient temperature. To assess the quality of impregnation, two pieces of net measuring 10 cm^2 were cut from different points and presented for chemical analysis using the HPLC at the Pyrethrum Board of Kenya (PBK) chemical laboratory, as previously described. In brief, before analysis, the pieces of net were weighed, soaked in 40ml of hexane in a beaker. The extract was shaken for 10min then transferred into a volumetric flask connected to a condensor where the sample was subjected to hot extraction of pyrethrins for 8 hours. After extraction, 50µL was injected into the HPLC machine for determination of the concentration of pyrethrins as previously described. All netting pieces analysed achieved a minimum of 95% of the target dose and thus ascertained the integrity of the impregnation process.

***Efficacy of pyrethrins-formulation impregnated on the three netting fabrics on knockdown and mortality of An. gambiae* s.l.**

After impregnation, the three different treated pieces of fabrics (i.e nylon, cotton and polyester) measuring 15cm^2 were cut and strapped onto one end of transparent plastic WHO cones using paper clips. About 20 female *Anopheles* mosquitoes, aged 3 days, were introduced into the cups and held in horizontal position so that the treated pieces of nets were in vertical position simulating the actual bed-net situation. The mosquitoes were then exposed to the nets for 3 min and the level of knockdown (KD) was monitored at 30 and 60 min post-exposure. Thereafter, the mosquitoes were transferred into holding cups and provided with sucrose (10%) pads and held in the recovery room for mortality assessment after 24 hours as per WHO procedure (WHO, 2006). Four replicate tests were conducted concurrently per treatment and fabric. Mosquitoes exposed to untreated nets or permethrin-treated nets at the recommended dosage were used as negative and positive controls, respectively. The bio-assay tests were conducted within one week after impregnation.

***Data Analysis*.**

Proportions were transformed to arcsine [ASIN(SQRT(n)] to normalize the distribution and stabilize the variance of the data. Analysis of variance (ANOVA) was carried out on transformed data to partition variation and interaction into components for fabrics, insecticides and dosage using SAS software. Means were separated using least significant difference ($t\acute{\alpha}/_2\ S\sqrt{2/n}$) at probability level of 0.05. Models of quadratic regression tests were generated to quantify the effect of the independent variables (i.e. insecticide dosage) on the dependent variables (i.e. knockdown and mortality levels), based on co-efficient of determination (r^2) values and lethal doses derived from the significant trend curves or lines. Statistical significance was assessed at a critical α-value of less than 0.05.

RESULTS

***Effect of pyrethrins-formulation impregnated onto different netting fabrics on bio-efficacy against An. gambiae* s.l.**

Results showed that, both fabrics and insecticide dose, were highly significant (P=0.0001) sources of variation in levels of knockdown and mortality of mosquitoes realized during the tests. A highly significant interaction between dose and fabric (P=0.0001) in effecting different levels of mosquito knockdown and mortality was also observed.
The pyrethrins-impregnated cotton nets showed significantly lower performance (P=0.0001) relative to polyester and nylon fabrics and multiple comparisons revealed that there was significantly higher knockdown and mortality with the nylon than polyester (P=0.0001, Table 1). The trend was similar with mortality of mosquitoes in the treated nylon fabric, though there were lower figures for overall mortality of 61.0% in the treated polyester net and only 14.1% in the treated cotton fabric (Table 1). In addition, there was a corresponding increase in knockdown and mortality of mosquitoes with increase in the dose of impregnated pyrethrins (Table 2). A higher knockdown and mortality of the mosquitoes was achieved when the nets were impregnated with pyrethrins at 1000mg/m^2 than at all the other treatment doses except for the standard permethrin dose of 500mg/m^2 (P=0.0001, Table 3). At 500mg/m^2, the pyrethrins-impregnated nets' performance was comparable to the standard permethrin-treated nets (Table 2). A significant interaction between the pyrethrins-treatment dosage and the treated fabric was observed (Table 3). However, for the impregnated nylon fabric, there was total efficacy of 100.0% mortality and knockdown at all the tested doses (Table 3).

Table 1: Effect of fabrics impregnated with pyrethrins on mean knockdown and mortality of *Anopheles gambiae* s.l.

Fabric	% KD-30 min ± SE	% KD-60 min ± SE	% Mortality ± SE
Nylon	90.41 ± 2.06[c] (1.256)	90.41 ± 2.06[c] (1.256)	90.41 ± 2.06[c] (1.256)
Polyester	73.57± 1.62[b] (1.031)	78.26 ± 1.79[b] (1.086)	61.03 ± 1.79[b] (0.897)
Cotton	22.26 ± 0.93[a] (0.491)	45.63 ± 1.09[a] (0.742)	14.10 ± 0.69[a] (0.384)
P-value	0.0001	0.0001	0.0001
LSD	0.0862	0.0604	0.0864

Data are means (± standard error) unless otherwise stated. Fabric refers to the three netting materials of nylon, polyester and cotton that were impregnated with pyrethrins-emulsifiable concentrate. Data in columns represented by %KD-30 and %KD-60 min show the proportions of mosquitoes that were knocked down after 30 and 60 minutes, respectively, following exposure to pyrethrins-treated nets for 3 min. % mortality refers to the proportion of mosquitoes that died after 24 hours post-exposure. Figures in brackets represent % values transformed into arcsine values. Means in same column with same superscript letter are not significantly different according to least significance difference (LSD) test at probability level of 0.05 based on transformed values. *P*-values represent the statistic probability levels.

Table 2: Effect of different doses of pyrethrins impregnated on nets on knockdown and mortality of *Anopheles gambiae* s.l mosquitoes

Conc. (mg/m^2)	% KD-30 min ± SE	% KD-60 min ± SE	% Mortality ± SE
0	0 .00 ± 0.00[a]	0 .00 ± 0.00[a]	0 .00 ± 0.00[a]
100	64.61 ± 3.24[b] (0.934)	83.71 ± 1.29[b] (1.155)	42.93 ± 3.79[b] (0.715)
250	80.78 ±1.74[c] (1.117)	82.56 ± 1.05[b] (1.140)	73.17± 2.43[c] (1.026)
500	83.89 ± 1.73[c,d] (1.158)	94.31 ± 1.07[b] (1.329)	81.64 ± 1.76[c,d] (1.128)
500p	90.28 ± 1.02[d] (1.254)	96.36 ± 0.68[b,c] (1.379)	94.19± 0.6[d] (1.327)
1000	97.8 ± 0.40[d] (1.422)	99.68 ± 0.14[d] (1.154)	95.65 ± 0.81[d] (1.361)
P-value	0.0001	0.0001	0.0001
LSD	0.1113	0.078	0.1115

Data are means (± standard error) unless otherwise stated. Data in columns represented by %KD-30 and %KD-60 min show the proportion of mosquitoes that were knocked down after 30 and 60 minutes, respectively, following exposure to pyrethrins-treated nets for 3 min. % mortality refers to the proportion of mosquitoes that died after 24 hours post-exposure. Figures in brackets represent % values transformed into arcsine values. Means in same column with same superscript letter are not significantly different according to least significance difference (LSD) test at probability level of 0.05 based on transformed values. *P*-values represent the calculated statistic probability levels.

Table 3: Interaction between pyrethrins dose and type of impregnated netting fabrics on mean knockdown and mortality of *Anopheles gambiae s.l.*

Fabric	Conc. (mg /m^2)	%KD-30min ± SE	%KD-60min ± SE	% mortality ± SE
Polyester	0	0.00 ± 0.0 ^{a}	0.00 ± 0.0^{a}	0.00 ±0.0^{a}
	100	75.00±0.06^{d,e} (1.0488)	86.22 ± 1.64^{c,d,e,f} (1.1905)	29.44 ± 0.16^{b,c} (0.574)
	250	87.34 ± 1.69^{d,e,f} (1.207)	79.30 ± 0.25^{d,e,f} (1.207)	78.71 ±4.45^{d,e,f,g} (1.091)
	500	94.24 ± 0.8^{e,f} (1.3285)	100.00 ± 0.0^{f} (1.570)	89.97± 1.6^{,d,e,f,g,h} (1.249)
	500P	94.98 ±1.95^{e,f} (1.345)	100.00 ± 0.0^{f} (1.570)	97.42 ± 0.85^{e,f,g,h} (1.4095)
	1000	100.00 ± 0.00^{f} (1.570)	100.00 ± 0.0^{f} (1.570)	100 ± 0.00^{h} (1.570)
Cotton	0	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}
	100	3.28 ± 3.28^{a,b} (0.182)	42.20± 0.22^{b} (0.706)	0.00 ± 0.00^{a}
	250	29.55 ± 0.3^{b,c} (0.574)	46.61 ± 0.06^{b} (0.752)	16.46 ± 0.11^{a,b} (0.418) ^{b,c}
	500	29.55 ± 0.3^{b,c} (0.575)	56.42 ± 0.32^{b,c} (0.849)	28.69± 0.29^{c,d} (0.565)
	500P	56.07 ± 0.23^{c,d} (0.846)	70.47 ± 0.26^{b,c,d,e} (1.002)	71.03 ± 0.05^{c,d,e,f} (1.002)
	1000	81.4 7 ± 0.06^{d,e,f} (1.126)	97.15 ± 0.96^{e,f} (1.401)	65.38 ± 0.18^{c,d,e} (0.941)
Nylon	0	0.00 ± 0.0^{a}	100.00 ± 0.0^{f} (1.570)	0.00 ± 0.0^{a}
	100	100.00 ± 0.0^{f} (1.570)	100.00 ± 0.0^{f} (1.570)	100.00 ± 0.0^{h} (1.570)
	250	100.00 ± 0.0^{f} (1.570)	100.00 ± 0.0^{f} (1.570)	100.00 ± 0.0^{h} (1.570)
	500	100.00 ± 0.0^{f} (1.570)	100.00 ± 0.0^{f} (1.570)	100.00 ± 0.0^{h} (1.570)
	500P	100.00 ± 0.0^{f} (1.570)	100.00 ± 0.0^{f} (1.570)	100.00 ± 0.0^{h} (1.570)
	1000	100.00 ± 0.0^{f} (1.570)	100.00 ± 0.0^{f} (1.570)	100.00 ± 0.0^{h} (1.570)
LSD		0.455	0.438	0.467
P-value		0.0001	0.0001	0.0001

Data are means (± standard error) unless otherwise stated. Fabric refers to the three netting materials nylon, polyester and cotton that were impregnated with pyrethrins-emulsifiable concentrate. Concentration (conc.) refers to the amount of pyrethrins impregnated on nets expressed in mg/m^2 except the standard permethrin at 500mg/m^2 designated as 500P. Data in columns represented by %KD-30 and %KD-60 min show the proportion of mosquitoes that were knocked down after 30 and 60 min, respectively, following exposure to pyrethrins-treated nets for 3 min. % mortality refers to the proportion of mosquitoes that died after 24 hours post exposure. Figures in brackets represent % values transformed into arcsine values. Means in same column with same superscript letter are not significantly different according to least significance difference (LSD) test at probability level of 0.05 based on transformed values. *P*-values represent the calculated statistic probability levels.

Additional results further demonstrated that the level of efficacy of a given dose was dependent on the fabric impregnated. For example, at 30 min post-treatment, a polyester fabric impregnated with equivalent dose of 100mg/m^2 of pyrethrins achieved 75.0% knockdown. This level was comparable to 250mg/m^2 (87.3% knockdown), 500mg/m^2 (94.2% knockdown) on the same fabric and to a treatment of 1000 mg/m^2 (81.4% knockdown) on pyrethrins-impregnated cotton fabric (LSD=0.455, *P*=0.0001). In terms of mortality, polyester net treated at 100mg/m^2 achieved 29.6% mortality, which was significanly higher than the 250-1000mg/m^2 impregnated on cotton fabric (*LSD*=0.467, *P*=0.0001).
To further determine the quantitative effect of treated fabric and impregnated dose on mosquito knockdown and mortality, regression tests were conducted and results (Table 4). The results show that the effect of impregnated dose on knockdown and mortality was not linear, but rather quadratic in nature as observed from the co-efficient of determination (r^2) values. The dose-response model in cotton fabric had relatively higher r^2 values of more than 0.90 relative to polyester fabric (r^2>0.75). However, nylon nets scored relatively lower r^2 values.

TABLE 4: Regression models of dose of pyrethrins impregnated on nets against knockdown and mortality of *Anopheles gambiae* s.l

Fabric	Regression equation(y = % KD-30min, x = mg/m^2)	r^2
Polyester	$y=21.67 + 0.277x - 0.00021x^2$	0.78
Cotton	$y = 4.33 + 0.058x - 0.0000174x^2$	0.96
Nylon	$y = 32.53 + 0.291x - 0.000229x^2$	0.59
	Regression equation(y = % KD-60min, x = mg/m^2)	
Polyester	$y=23.83 + 0.283x - 0.00021 x^2$	0.75
Cotton	$y=12.94 + 0.140x - 0.000124 x^2$	0.88
Nylon	$y=32.53 + 0.291x - 0.291 x^2$	0.51
	Regression equation(y = % mortality, x = mg/m^2)	
Polyester	$y=4.63 + 0.269x-0.00017 x^2$	0.75
Cotton	$y=0.04+ 0.15x-0.000051 x^2$	0.99
Nylon	$y=32.53 +-0.29-0.000229 x^2$	0.59

Fabric refers to the three netting materials nylon, polyester and cotton that were impregnated with pyrethrins-emulsifiable concentrate. Regression equations are the models that describe the unit effect of the independent variable, concentration (X) and dependent or response variables, knockdown (%kd-30min, %kd-60min) and mortality depicted by *y*. The r^2 represents the coefficient of determination showing what quantity of variation is explainable by the equation.

In order to describe the effect of unit increase in dose on knockdown and mortality of the test mosquitoes, regression coefficients were additionally carried out (Table 4). At the linear portion of the quadratic equation, there was higher increase per unit dose in treated nylon and polyester fabrics, than in cotton fabric. For example, at 30 min post-exposure to the pyrethrins-impregnated nets, a unit increase in dose resulted in 0.291% ($y=32.53 + 0.291x-0.000229x^2$) increase in knockdown in nylon nets, 0.277% ($y=21.67 + 0.277x-0.00021x^2$) in polyester net and only 0.058% ($y=4.33 + 0.058x-0.0000174x^2$) in cotton fabric (Table 4). However, beyond the linear portion of the quadratic equation, there were subsequent negative regression coefficients with a higher decrease in nylon netting fabric than in polyester and cotton (Table 4). The non-linearity of the dose-response observed in the presented data shows that increase in dose of pyrethrins-impregnated on a net is not directly proportional to the increase in bio-efficacy. In essence, there was a higher unit increase per dose realized in pyrethrins-impregnated nylon, with the least increase being observed in the treated cotton fabric.

The dose of pyrethrins-emulsifiable concentrate that achieved 95% knockdown of mosquitoes (Kd_{95}) after 60 min post 3-min exposure on polyester fabric was 350mg/m^2 and 275mg/m^2 on nylon (Figure 1). In terms of effecting 80% mortality (LC_{80}) after 24 hours, polyester fabric required 375mg/m^2 as compared to only 200mg/m^2 for nylon fabric (Figure 2). In contrast, cotton fabric required a much higher dose of insecticide in order to achieve the 80% lethal value (Figures 1 and 2).

Figure 1: *Predicted knockdown of Anopheles gambiae* s.l. *exposed to pyrethrins treated netting fabrics at different doses.* The three netting materials nylon, polyester and cotton were impregnated with pyrethrins-emulsifiable concentrate. Kd_{95} refers to the calculated dose of pyrethrins emulsifiable concentrate that achieved 95% knockdown of mosquitoes after 60 min following exposure of mosquitoes to pyrethrins-treated nets for 3 min. For the polyester fabric, it was extrapolated to be 350mg/m^2 based on the regression equation $y = 4.63 + 0.269x - 0.00017x^2$ and for nylon it was 275mg/m^2 $y = 32.53 +-0.29 - 0.000229x^2$ where y is predicted % knockdown and x is concentration of pyrethrins impregnated on a net in mg/m^2.

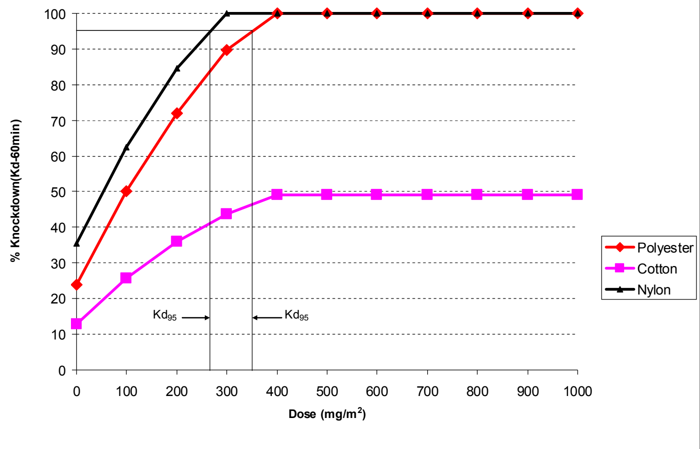

Fig.1: Predicted Knockdown of *Anopheles gambiae* s.l. exposed to pyrethrins treated netting fabrics at different doses.

Figure 2. *Predicted mortality of Anopheles gambiae* s.l. *exposed to pyrethrins treated netting fabrics at different doses.* Nylon, polyester and cotton were impregnated with pyrethrins emulsifiable concentrate. LC_{80} is a calculated dose that achieved 80% mortality of test mosquitoes after 24 hours following 3 min exposure to pyrethrins-treated nets. For polyester fabric, it was 375mg/m^2 extrapolated from the regression equation $y = 4.63 + 0.269x - 0.00017x^2$ and for nylon it was 200mg/m^2 based on regression equation $y = 32.53 +0.29 - 0.000229x^2$ where y is predicted % mortality and x is concentration of pyrethrins impregnated on a net in mg/m^2.

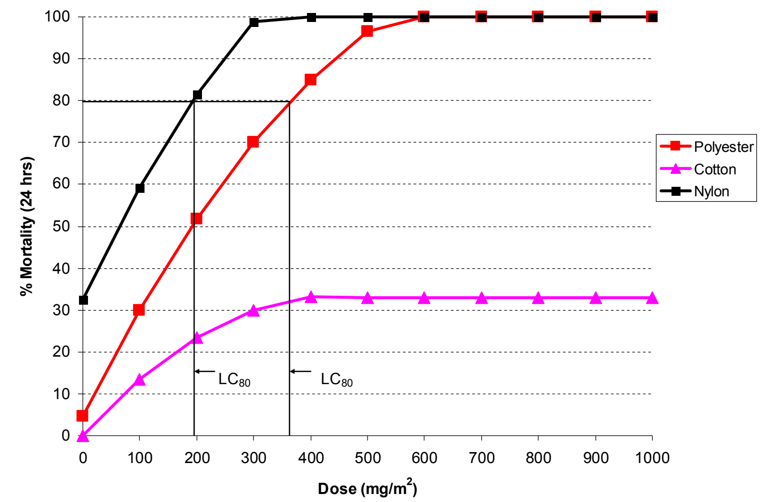

Fig.2: Predicted kill of *Anopheles gambiae* s.l. exposed to pyrethrins treated netting fabrics at different doses.

DISCUSSION

Appropriate choice of a netting fabric for use in ITNs is crucial and is closely related to the compatibility of the fabric with insecticide formulation (Rozendaal, 1989; WHO, 2006). In the current study, it has been demonstrated that pyrethrum formulation work best on nylon, followed by polyester, with the worst bio-efficacy observed in the cotton material. The relatively good compatibility (i.e. bio-efficacy) of the pyrethrum formulation in the synthetic nylon and polyester fabrics is probably, due to the fact that the polymer chains in nylon and polyester have the same polarity as pyrethrins (Casida and Quistard, 1995), resulting in limited antagonistic activity. The observed differences in bio-efficacy of the different insecticide-treated netting fabrics in the current study are consistent with previous findings on pyrethroids-impregnated nets (Vatandoost *et al.,* 2006). In these previous studies, there was relatively poor bio-efficacy on treated cotton nets, although the insecticide formulation performed better in polyester fabric than in nylon. The high level of knockdown experienced with the treated nylon net at all doses in the current study supports previous findings in which a low dose of 25mg/m^2 of Cyfluthrin and Deltamethrin, impregnated on nylon net, resulted in 100.0% mortality on female mosquitoes that landed on the fabric (Ansari and Razdan, 2000). However in a different study, it was observed that polyester achieved a better bio-efficacy than nylon thus underscoring the importance of the nature of a formulation on the bio-performance of an impregnated material (Vatandoost *et al.,* 2006). The poor performance of the impregnated cotton nets observed in the current and previous studies are probably due to the texture of this fabric (i.e. it is rough, porous and absorbent). These combined properties allows uptake of more insecticide than the synthetic materials, however, a substantial part of the insecticide is not contactable on the surface resulting in the possibility of the mosquitoes picking up sub-lethal doses (Lines *et al.,* 1987; Rozendaal, 1989). In addition, cotton nets contain starch which dissolves during impregnation and this phenomenon can affect the binding and bio-availability of insecticide for contact by the mosquito (Nadanathangam *et al.,* 2006).

We observed significant interaction between insecticide dose and fabric in the two-way ANOVA results, pointing out that the performance of a given dose of insecticide was not uniform among the fabrics. This is further confirmed by the regression models that showed higher regression coefficients in the synthetic fabrics of nylon and polyester than in the natural cotton fabric. The relatively higher coefficient of determination values of more than 0.90 in the quadratic model observed in the treated cotton fabric show that the effect of dose was heightened to 90.0% in the equation while it was only 75.0% for polyester and 56.0% for nylon. The KD_{95} and LC_{80} values on knockdown and mortality show that a much higher dose of insecticide is needed to impregnate cotton nets in order to realize the same effect as polyester and nylon (Figures 1 and 2). This has been demonstrated in previous studies in which it was observed that the texture of a net determines the target insecticide concentration necessary for its impregnation (Rafinejad *et al.,* 2008). This suggests that insecticide-impregnated cotton net would be more costly and further have an adverse impact on demand of the material, resulting in less usage. Affordability of a product is normally positively correlated with its acceptance by users. Insecticide-impregnated net that is relatively inexpensive would, therefore, earn consumers preference thus contributing to the success of malaria control effort. It has been observed that in the South-East Asian countries, local availability of cheap mass-produced mosquito nets enhances large-scale use of ITNs, while in Africa and South American countries, the higher prices of locally-made mosquito nets hamper large-scale use (Rozendaal, 1989). Besides, the relatively lower impregnation dosage required for synthetic nylon and polyester fabrics, makes them cheaper, and more preferable than cotton nets (Rozendaal, 1989).

The fact that at an equivalent dose, the pyrethrins-formulation under evaluation was comparable to the standard permethrin at the recommended concentration of 500mg/m^2 confirms the suitability of the pyrethrins-formulation for use in impregnated nets. Pyrethrins-formulation is based on a natural product (as the active ingredient) and is environmentally-friendly and, therefore, presents an opportunity for rational use of insecticides in malaria control (Warui and Mutinga, 1994). The inclusion of an anti-oxidant in the formulation will help retard atmospheric oxidation or the degenerative effects of oxidation on the pyrethrins, thus stabilizing the molecule to last long on the treated net (Casida and Quistard, 1995). The synergist included in the formulation is thought to act through competitive metabolic enzyme inhibition, alteration of cell membrane or rendering of receptor site accessibility to the active compound. This provides more potential to fight resistance since synergists have been shown to also enhance stability of pyrethrins (www.endura.it).

In conclusion, the current study confirms the potential for use of the pyrethrins formulation in ITNs. This finding is significant since vector resistance to synthetic pyrethroids which has previously been reported (Hargreaves *et al.,* 2000; Ranson *et al.,* 2000) may at one time call for "boosting" of the nets through re-treatment and hence availability of suitable and effective formulations is crucial. Furthermore, the study demonstrated that treatment of nets should take into consideration the nature of fabrics and impregnation dose to maximize on the bio-efficacy potential of a net. The pyrethrins-formulation was most suitable for treatment of nylon and polyester nets while its use in cotton may prove un-economical. Without any anticipated degradation due to washing and photolysis, the empirically derived dose of pyrethrins for use in polyester fabrics would be about 375-400mg/m^2 which yields >95% knockdown and >80% mortality, as per the WHO specification. Based on the same criteria, a lower dose of 250mg/m^2 would be recommended for nylon, while a dose of >1000 mg/m^2 would be appropriate for cotton fabric. These observations are useful in the choice of nets for use in places of high endemic mosquito populations, where they serve both as disease vectors and a nuisance to the population. However, there is need for further studies to determine how the fabrics and dosage affect key behavioral responses such as blood feeding inhibition and repellency of mosquitoes.

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