LABORATORY PROTECTION RATE OF TORN BEDNETS TREATED WITH THREE DOSAGES OF PYRETHROIDE AGAINST ANOPHELES CULICIFACIES

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Abstract- For the prevention of human malaria, vector control is an important component of the global strategy. The objective of the present study was to observe the effect of impregnated torn bednets with three dosages of cyfluthrin 5% on the number of bites by Anopheles culicifacies. A glass made tunnel test was designed and hungry female mosquitoes were induce to pass through holes cut in the pyrethroid treated nets. A guinea pig used as bait to attract mosquitoes through circular holes in the netting. With untreated netting, 72-87% of laboratory-reared females passed through the holes overnight and 64-92% blood-fed successfully. When the netting was treated with cyfluthrin at doses of 25, 50 and 100 mg a.i./m², the entry index (the proportions that passed through the holes overnight) were 43.37%, 42.82% and 24.72%; mortality rates were 66.31%, 81.45% and 95.99%; and the feeding rate were 45%, 27% and 3%, respectively. Pyrethroid impregnated bednets using “tunnel tests” showing acceptable protection rate both in lower and higher dosages. In addition, the higher dosages of these three dosages pyrethroid provided good levels of protection against An. culicifacies.

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Key words: Anopheles culicifacies, torn impregnated nets, cyfluthrin, Iran

INTRODUCTION

For the prevention of human malaria, vector control is an important component of the global strategy (1). Vector control aims to prevent the transmission of malaria parasites by methods such as house spraying with residual insecticides (2) or personal protection by the use of pyrethroid-impregnated bednets (3). Since Herodotus travel to Egypt, the bednets has assumed an important role in the control of malaria (4). Anti malaria bednets, treated with DDT, were first used during World War II by the Russian, German and US armies (5) but it was not until the 1980’s that controlled trials demonstrate significant protection against the incidence of malaria (6). Seasonal factors, patterns of use and question of cost are key factors likely to influence the effectiveness of bednets (7). Pyrethroid-treated bednets have been shown in recent trials to have an important impact on cases of malaria (8), incidence of infection and prevalence of anemia (9) and all-caused child deaths in several parts of the Africa (10). Treated nets are more acceptable and affordable than house spraying (9). The acceptance and usage of treated bednets is better than ordinary bednets (11). DDT spraying was more than six times
more expensive per person per year than providing the bednets (2).

Bednets in perfect condition can prevent 90% of bites (2), but they are usually so damaged that mosquitoes are not stopped from taking a blood meal on the sleepers. A number of surveys have recently demonstrated the efficacy of torn bednets on reduction of man-vector contact. If a considerably torn bednet correctly be impregnated, it could be a useful method for limiting human-anopheline contacts (12). Damaged bednets with a large number of holes and impregnated with the pyrethroid etophenprox or organophosphate (OP) pirimiphos methyl did not provide protection against being bitten by pyrethroid resistance An. gambiae and Culex spp. (9).

The netting barrier under test was made of polyester with holes of standard size cut in it to simulate a torn bednet, as is often found in rural tropical houses. This has been used to test a series of different dosages of cyfluthrin, deltamethrin and permethrin, which have been developed by the pesticide industry for bednet impregnation.

**MATERIALS AND METHODS**

**Mosquitoes**
Mosquitoes were reared to the pupal stage on a diet of BEMAX®. Adults were kept at \(75\pm5\%\) RH and \(28\pm2\,\text{C}\) in a photophase of 12:12 (L: D) h with unlimited access to 10 glucose.

**Pyrethroid insecticides**
Tests were performed with cyfluthrin 5% EW, obtained from Bayer®. A range of three concentrations of each of these pyrethroid was used to examine the relationship between dose and response.

**Impregnation**
The netting was impregnated by immersion in a mixture of insecticide and water in any dose. The netting samples were dried in the laboratory. Testes were carried out 24 h after impregnation. The amount of insecticide required for treatment of the netting was calculated using the following formula:

\[
\text{Recommended dosages} \times \text{are of net/concentration of insecticide} \times 10 \times 100
\]

The volume of water absorbed by the samples of polyester was calculated by comparing the weight of five samples of each net when dry and when wet (dipped in water and excess water squeezed out).

**Tunnel Test**
Polyester 100 denier multifilament netting was tested to determine whether An. culicifacies females were able to pass through the holes having 1 cm diameter in sheets of treated or untreated netting, and whether the mosquitoes would take a blood meal there after with each of insecticides.

Testes were performed in a square glass “tunnel” (height 25 cm, width 25 cm, length 60 cm) with cage ends, as described by Elissa and Curtis (5) and Chandre (13), subdivided changeable piece netting with 9×1 cm holes (1% of total area) inserted on a cardboard frame across the tunnel. Netting was previously impregnated with insecticide to give a predetermined treatment rate (mg ai/m²). After drying the impregnated piece of net used for testing 24 h post impregnation. In one end of the tunnel a guinea pig was placed as bait, held in a small metallic holder to prevent contact with the netting. In the other end of the tunnel, \(100\) unfed female mosquitoes (6-8 days old) were introduced at 17:00 hours and the apparatus was left overnight in a dark room maintained at 28º C and 80% RH. The next morning, at 07:00 hours, the numbers of mosquitoes in both compartments were counted and their mortality and blood-feeding rates were scored. Testes were replicated three times for each dose, EW 5% cyfluthrin 25, 50 and 100 mg/m², and control untreated netting.

**Statistical analyses**
Data was analyzed by observed percentage mortality were corrected by Abbott’s formula for contemporary control mortality. For significant testing, corrected mortalities were Arcsine transformed and submitted to one-way ANOVA in SPSS.

A value of \(P \leq 0.05\) was considered statically significant.
RESULTS

For three dosage of insecticide (cyfluthrin) tested in tunnel cage, mortality rates ranged from 3.9% to 4.34% with non-impregnated netting (Table 1). The proportions of mosquitoes that succeeded in passing through holes in the net (entry index) were from 77% to 99% and taking a blood meal (individual protection) from 64% to 92%.

Effects of impregnated torn nets on the numbers of mosquitoes entering from holes in three assessed of cyfluthrin are shown in Fig. 1. About 38% fewer An. culicifacies entered the tunnel containing cyfluthrin treated torn nets than untreated torn nets ($P < 0.01$). There were no significant differences between the numbers of An. culicifacies in treated torn nets with permethrin and cyfluthrin. In cyfluthrin (at 25 mg/m$^2$) about 25% of mosquitoes were succeeded in passing through holes ($P < 0.05$).

We observed about 100% mortality in high dosage of cyfluthrin (100 mg/m$^2$); therefore the test did not carried out with high dosage of this insecticide (1000 mg/m$^2$) (Table 1). For An. culicifacies with the torn nets, all the insecticides caused significantly greater mortality than the untreated torn nets ($P < 0.01$). There was significant difference between the numbers of An. culicifacies killed by cyfluthrin and control in low dosage. The highest percentage of mosquitoes was killed with cyfluthrin in 100 mg/m$^2$ (95%) concentration. In low dosages, fewer An. culicifacies was killed, but all of them killed more mosquitoes than untreated torn nets. The tunnel with untreated nets had a significantly higher percentage of blood feed females than any of treated nets ($P < 0.01$).

With cyfluthrin treated torn nets, there were significant differences in the proportions of blood feed with control in An. culicifacies females in low and medium dosages. Low dosage of cyfluthrin offered 20% individual protection, whereas it was 60% in control. The best individual protection against An. culicifacies was obtained with cyfluthrin at 100 mg/m$^2$ (nearly 100%) concentration (Fig 2).

In high dosage, cyfluthrin treated torn nets had a significantly lower percentage of blood feed females than control ($P < 0.01$).

DISCUSSION

Prevention of mosquito probing and blood feeding is a doubly important function of treated bednets, directly reducing transmission risk and allowing a good night sleep. With untreated bednets, mosquitoes can probe through the net or enter through torn holes to bite the sleeper.

Kolaczinski et al. reported that with increase of dosage of cyfluthrin, blood feeding is decrease (9). Yadava et al. stated that cyfluthrin impregnated bednets reduced blood feeding of mosquitoes (14). Present study is the first report from application of cyfluthrin against An. culicifacies in torn nets. In our study, in 100 mg/m$^2$ dosage of cyfluthrin observed the best efficacy in individual protection. Low dosages of this insecticide (25 mg/m$^2$) can reduce entry index, blood feeding and increase mortality rate of An. culicifacies in comparison with control (>80% reduced blood feeding). When cyfluthrin used, the trend of mortality rate was greater when the insecticide concentration was increased.

Table 1. Effect of netting impregnated with cyfluthrin 5% EW in tunnel cage on An. culicifacies

<table>
<thead>
<tr>
<th>Insecticide Doses</th>
<th>Group</th>
<th>N</th>
<th>Mortality (%)</th>
<th>Passed through net (%)</th>
<th>Blood-fed</th>
<th>Survival rate</th>
<th>PI</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg/m$^2$</td>
<td>control</td>
<td>285</td>
<td>4.21</td>
<td>77</td>
<td>204</td>
<td>78</td>
<td>72</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>test</td>
<td>279</td>
<td>66.31</td>
<td>43</td>
<td>45</td>
<td>23</td>
<td>15.6</td>
<td>0.13</td>
</tr>
<tr>
<td>50 mg/m$^2$</td>
<td>control</td>
<td>282</td>
<td>3.9</td>
<td>85</td>
<td>182</td>
<td>89</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>test</td>
<td>275</td>
<td>81.45</td>
<td>42</td>
<td>27</td>
<td>15</td>
<td>0.1</td>
<td>0.06</td>
</tr>
<tr>
<td>100 mg/m$^2$</td>
<td>control</td>
<td>276</td>
<td>4.32</td>
<td>93</td>
<td>255</td>
<td>96</td>
<td>92</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>test</td>
<td>271</td>
<td>95.99</td>
<td>24</td>
<td>3</td>
<td>0.04</td>
<td>0.1</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: N, number; PI, individual protection.
Individual protection with this insecticide was 20-80%, depend on the dosages. Torn bednets impregnated with cyfluthrin (at 25 or 50 mg/m²) reduced by nearly 50% the contact between humans and An. gambiae (15). In our study, torn impregnated nets reduced 50% contact between An. culicifacies and guinea pig at 50 mg/m². In An. culicifacies, in contrary with An. gambiae (12) mortality rate was increased when the insecticide was increased.

Tests of torn nets impregnated with cyfluthrin revealed the best individual protection (> 90%) in our study. It has been reported that torn bednets impregnated with 500 mg/m² of cyfluthrin reduced 60% entry of An. Gambiae (15), which reduced 80% of entry of An. culicifacies at same dosage in our study. Mortality rate of An. culicifacies exposed to cyfluthrin treated nets was dependent to insecticide concentration.

Different insecticides showed various degrees of irritancy and killing ability against mosquitoes when used for net impregnation (5). The results of this study indicate that different dosages of tested insecticides showed various degrees of irritancy and killing ability against An. culicifacies, when used for impregnation of torn nets. Pyrethroid-impregnated bednets in tunnel tests, showing that if insecticide and its dosage are selected properly, even torn bednets impregnated with pyrethroid provided good levels of protection against An. culicifacies.

Conflict of interests
The authors declare that they have no competing interests.

REFERENCES


