DISCUSSION
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1. Bioassay

The results of cone bio assay in the present investigation confirms the efficacy of pyrethroid treated nettings against all the mosquito species evaluated against them and the results are in conformity with Ansari (2002) who have reported the maximum efficacy of Etofenprox treated fabrics against susceptible *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. Hougard *et al.* (2002) has reported 100% mortality of *Culex* quinquefasciatus and *Anopheles* gambiae when the usefulness of Bifenthrin was assessed to be an effective candidate for net treatment. Ansari *et al.*, (2003) studied the feasibility of Alpha cypermethrin nets in controlling mosquitoes and found that initial performance of treated nettings were appreciable against *Anopheles culicifacies* which is in line with the present investigation. Curtis *et al.* (1996) compared the efficacy of different insecticide treated nettings and Fabrics and found that Permethrin bed nettings gave better personal protection against susceptible *Anopheles* and *Culex*. Graham *et al.* (2004) recorded 100% knockdown after 3 minutes of exposure on Deltamethrin treated tents, which confirms the results of the present investigation. Kapoor and Ansari (2003) studied the efficacy of synthetic pyrethroids, on cotton fabrics and reported that Deltamethrin was the most effective exhibiting 100% mortality against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* which falls in line with the present investigation. The reason for this marginal inferior performance shall be attributed to the fabric evaluated on. Vythyalingam *et al.* (1996) reported 100% mortality of *Anopheles maculates* and *Aedes aegypti* on Olyset net which confirms the results of present investigation. Jawara *et al.* (1998) studied the efficacy of Permethrin, Lambda cyhalothrin and
more effective than the washed nets and efficacy decreases with washing which is similar to with the present finding. Elissa and Curtis (1995) reported that Deltamethrin formulations performed better than the Permethrin in terms of mosquito mortality and the same is observed in the present investigation.

The resistant *Anopheles* showed remarkably less mortality and knockdown on all the insecticide treated netting. Similar results was observed by Hodjati *et al.* (2003) when Permethrin (200 mg/m²), Deltamethrin (25 mg/m²) treated nettings evaluated against resistant and susceptible *Anopheles stephensi*.

2. **The median time knockdown**

The Median time knockdown was faster in the unwashed nettings and was found to decline with washings. The fact fall in line with Yates *et al.* (2005) who reported 2.4 folds increase in MTKD with conventional nets after 30 washings. The resistant *Anopheles* were found to recorded a higher MTKD values than the susceptible *Anopheles* and the result of the present study is in line with Hodjati *et al.*, (2003) with Permethrin (200 mg/m²) and Deltamethrin (25 mg/m²) treated nettings against resistant and susceptible *Anopheles stephensi*. Graham (2005) reported used sensitive MTKD and proved loss of efficacy in conventional nets with washings and this is in line with the findings of the present investigation.

3. **Irritant effect**

In the present study, Lambda cyhalothrin proved to be a better irritant than Permethrin. But as per findings of Hodjati *et al.* (2003) Permethrin proved to be a better irritant against susceptible *Anopheles* than Lambda cyhalothrin, which is inconsistent with the present findings. Irritancy exhibited to susceptible species was
better than to the resistant *Anopheles* and the same was observed by Hodjati *et al.* (2003) against resistant *Anopheles*.

*Vatandoost* (2001) reported Lambdacyhalothrin a better irritant followed by permethrin for susceptible *Anopheles stephensi*. However, in the present investigation, Permethrin showed better irritant effect against susceptible *Anopheles* followed by Lambda cyhalothrin and Deltamethrin. Evans (1993) reported that Lambda cyhalothrin as a very good excito repellent against *Anopheles gambiae* and similar observations are found in the present investigation. Hougard *et al.* (2003) has reported Etofenprox, Permethrin Deltamethrin and Lambda cyhalothrin as better irritant followed by Bifenthrin against susceptible *Anopheles gambiae*. Hodjati *et al.* (2003) reported an increased $FT_{50}$ values with resistant *Anopheles stephensi* when evaluated against Permethrin, Alphacypermethrin and Lambda cyhalothrin treated nettings. Similar observations were observed in the present investigation with *Anopheles stephensi*. Hougard *et al.* (2003) reported that for susceptible *Culex quinquefasciatus* irritant effects did not greatly differ among insecticides, which are not in line with the present investigation. Alipour *et al.* (2006) reported that Deltamethrin treated bed nettings exhibited better excito repellency against susceptible *Anopheles stephensi* than Lambda Cyhalothrin, which is reverse of the finding of present investigation. Ansari and Razdan (2000) studied the efficacy of Deltamethrin, Cyfluthrin, Lambdacyhalothrin, and Etofenprox at 25 mg/m2 treated nylon net and reported 100% mortality of landed *Culex quinquefasciatus* and *Anopheles*, which is in line with the present study. The repellent effect was more pronounced against *Anopheles* to *Culex* which confirms the present finding. Bifenthrin to be less irritant than Permethrin and Deltamethrin, even with *Cx. quinquefasciatus*, which is more
susceptible to the irritant effect of pyrethroids and has reduced knockdown (WHO, 2001), which confirms the results of the present investigation.

4. Blood Feeding Inhibition

In the present investigation, the test nettings effectively prevented the blood feeding by the susceptible mosquito species tested. Hodjati et al. (2003) reported higher BFI for Permethrin followed by Deltamethrin for *Anopheles stephensi* and are not in line with the present study. However, nettings recorded poor BFI against resistant *Anopheles*, which is in line with the present investigation (Hougard, 2002). Vythalingam et al. (1996) studied 95 and 85% penetrability of *Aedes* and *Anopheles*, which does not fall in line with the present finding. However, 100% mortality of all landed insects confirms the results of the present finding. Asidi et al. (2005) reported that the efficacy of Lambda cyhalothrin treated nets to inhibit blood feeding is effective up to five washes against *Culex* and *Anopheles*, which does not confirm with the result of the present study. Asidi et al. (2004) studied various formulations of Permethrin. Alpha Cypermethrin, Deltamethrin and Lambda cyhalothrin and reported that unwashed nets significantly reduced the blood feeding which is in line with the finding of present study. Guessan et al. (2001) reported strong deterrency of Olyset net to resistant *Anopheles gambiae* and is in line with the present findings where olyset recorded higher BFI than the conventionally treated nettings. Curtis et al. (2001) in verandah-trap study in Tanzania reported that conventionally treated with Permethrin gave similar results to the Olyset net in terms of blood feeding, which confirms the present study.
5. Storage

Moosa-Kazemi (2007) reported that Bioassays on polyester nets treated with Deltamethrin showed persistence of almost 100% mortality of Anopheles stephensi on 7 months old net, which is in line with present finding. The field trial on the efficacy of a suspension concentration formulation of Deltamethrin against malaria vectors showed generally comparable results with a similar trial undertaken in the same area (Yadav et al., 2001). Batra et al. (2005) studied different formulations of Bifenthrin and reported the bio availability for five months and confirm the result of present investigation. Nguyen et al. (1996) reported 100% mortality of Aedes aegypti for eight months and the same is confirmed in the present investigation.

6. Washing

Itoh and Okuno (1996) reported that persistence of Permethrin in Olyset net was examined after seven months of practical use and it confirms the present finding as no appreciable difference was observed in the surface concentration of Permethrin. This is well reflected in the consistent results of bioassay, BFI and irritancy exhibited by Olyset net against the mosquito species tested. Ikeshoji and Bakotee (1997) reported that 76% of Permethrin is lost after a single wash and regained 80% after drying in sun and is consistent with the present finding. WHO (1999) concluded that Etofenprox deposits are more wash resistant than Permethrin and it is confirmed in the present result as Etofenprox treated nettings registered higher mortality, repellency and BFI than the Permethrin treated netting. Graham (2005) reported the quick loss of insecticides with conventionally treated nets and this confirms the findings of the present study.
The overall perusal of data reveals that the efficacy exhibited by conventionally treated nettings are comparable with Olyset net when assessed unwashed. But with washings the efficacy in terms of knockdown, mortality, irritancy and BFI was found to reduce appreciably. The reduction in the insecticide concentrations with washings may be attributed as a valid reason for the poor performance of the conventionally treated nettings.

The efficacy of the nettings against the F3 generation of resistant *Anopheles* underlines the fact that the susceptible level of F3 generation does not differ significantly from the parent population. However, The resistant adults of F12 generation was challenging to the conventionally treated nettings irrespective of the insecticide treated (Figure 13 - 16). Lower knock and mortality, decreased BFI and less irritancy proved their resistant status Figure 40 & 41). But, the mortality exhibited by the resistant individuals may be due to the fact that they could have got exposed to insecticide for a longer time owing to less irritancy and the concentration would have been sufficient to exhibit mortality.

Olyset, a long lasting insecticide treated net, in which bio-availability of the insecticide is at the surface of the fiber. Through this process, the residual efficacy is longer than that of conventionally treated nets. Since only a small fraction of Permethrin is bio-available at the surface of the fibre at any time, and there is no need for treatment at peripheral level, the use of Olyset net is even safer than that of conventionally treated nettings. There is evidence that Olyset nets are as effective as nets conventionally treated with Permethrin in killing vector mosquitoes, reducing blood feeding, as well as having an excito-repellent effect. They also have significantly longer residual efficacy. A major advantage of Olyset
Figure 13. Comparison of KD_{60} of resistant and susceptible *Anopheles stephensi* on unwashed nettings
Figure -14. Comparison of KD$_{60}$ of resistant and susceptible *Anopheles stephensi* after three washings.
Figure - 16. Comparison of Mortality of resistant and susceptible *Anopheles stephensi* after three washings.
Figure -41. Comparison of BFI of resistant and susceptible *Anopheles stephensi* after three washings.
Long Lasting Net is that its biological efficacy is resumed by diffusion of the insecticide from the inside of the yarn to the surface (WHO, 2001). Thus the surface concentration of Permethrin is maintained which is sufficient to act against susceptible mosquito population. Though Olyset proved to be better candidate against susceptible mosquito species, a reduction in efficacy was obvious with F12 Permethrin resistant adults. However, the efficacy of Olyset net against resistant adults was significant when compared with conventionally treated nettings.

7. **Acute Oral toxicity**

Permethrin is moderately to practically non-toxic via the oral route, with a reported LD$_{50}$ for technical Permethrin in rats of 430 to 4000 mg/kg (Extox net, 1994) and this confirms the results of the present investigation. Permethrin's LD$_{50}$ (the amount of Permethrin that kills 50 percent of a population of test animals) is variable. WHO reported that in a summary of nine oral LD$_{50}$ tests using rats, the LD$_{50}$ varied from 430 milligrams per kilogram of body weight (mg/kg) to over 4,000 mg/kg and this in line with the present findings.

8. **Skin sensitisation**

Flannigan and Tucker, 1985; Flannigan *et al.*, 1985a,b reported that mild skin irritation in people whose skin was exposed to Permethrin, at 0.13 mg/cm$^2$, lasting less than 24 hours. Schultz *et al.* (1990) reported mild itching as well as temporary burning or stinging of the skin in response to application of topical 5 percent Permethrin-containing creams to treat scabies. Soto *et al.* (1995) in a study of Permethrin-impregnated uniforms in Cambodian soldiers, reported that Permethrin is relatively non-toxic when impregnated in clothes, as only 2 of the 229 soldiers reported mild skin irritation.
In the present study, no observable effect on skin was observed in spite of continuous exposure of the olyset net for 14 days (8 hours/day). The safety exhibited by the test net may be due to the very low surface concentration of Permethrin (26.95 to 28.57 mg a.i/m2). Andrews et al. (1992) reported that the rate of increased skin rashes and eye irritation was 2.2 per 1000 treatments among patients treated with Permethrin. No serious, unexpected adverse events were detected in the 18,950 patients treated with Permethrin. This confirms the fact that Permethrin is safe to human skin and contact of very low concentration of Permethrin has little or no effect on the skin. Flannigan (1985a) reported that repeated daily applications of either Fenvalerate or Permethrin to albino rabbits, caused slight erythema, which was correlated with increased cutaneous blood flow and it is not in line with the findings of the present study.

Human Health Monitoring study

The health monitoring study did not reveal overt signs of toxicity among the users or inhabitants. No hematological, biochemical and allergic reactions were noticed among the users of LLIN.

Yadav (1995) have reported headache, skin irritation, burning sensation in the eyes, lacrimation and nausea among the insecticide treated bed net users and these symptoms were considered to be transient. No such effect was reported by the users of the LLIN in the present study during the informal enquiry made during the study course.

Satpathy et al. (1997) reported no effect on health or laboratory impairment in the subjects after one month of usage of nets impregnated at the dose of 50 mg/m2 of
cyfluthrin and is in line with the results of the present study using LLIN. The levels of exposure of these subjects were not measured in both the studies.

In a room where Cyfluthrin (50 mg Cyfluthrin/m2) treated net was hung, mean cyfluthrin levels 10 cm inside the net ranged from 0.02 to 0.055 µg/m3, while concentrations outside the net were slightly lower. The difference was attributed by the author to a better air exchange (Boman, 1995). Barlow et al. (2001), through extrapolation from the highest concentration observed by Boman (0.055 µg/m3), reported that that the estimated inhalation exposure is significantly lower and stated that the risk for bednet users is acceptable. In the present study the Permethrin concentration was measured neither in air nor in blood samples as the surface concentration of Permethrin is very low. The concentration of Permethrin in the zone of inhalation would have got diluted further by air exchange. Hence the concentration of Permethrin both in air and blood was not assessed.

As the risk assessments carried out by Boman, and Barlow et al was in the worst-case scenario, the conclusions could be extrapolated for the whole group of pyrethroids recommended by WHO for bednet treatment. Wilks (2000) concluded that, if pyrethroids are used with the recommended precautions, no major symptoms of poisoning, other than paraesthesia, can be expected in bed net users. Since paraesthesia is a transient and rather common symptom of low dose exposure to pyrethroids, limited to the site of exposure, and can be considered a nuisance effect rather than a symptom of severe toxicity, no major health risk is anticipated for bed net users.

The present study confirms the same showing no health effect among the users of LLIN during the study period.